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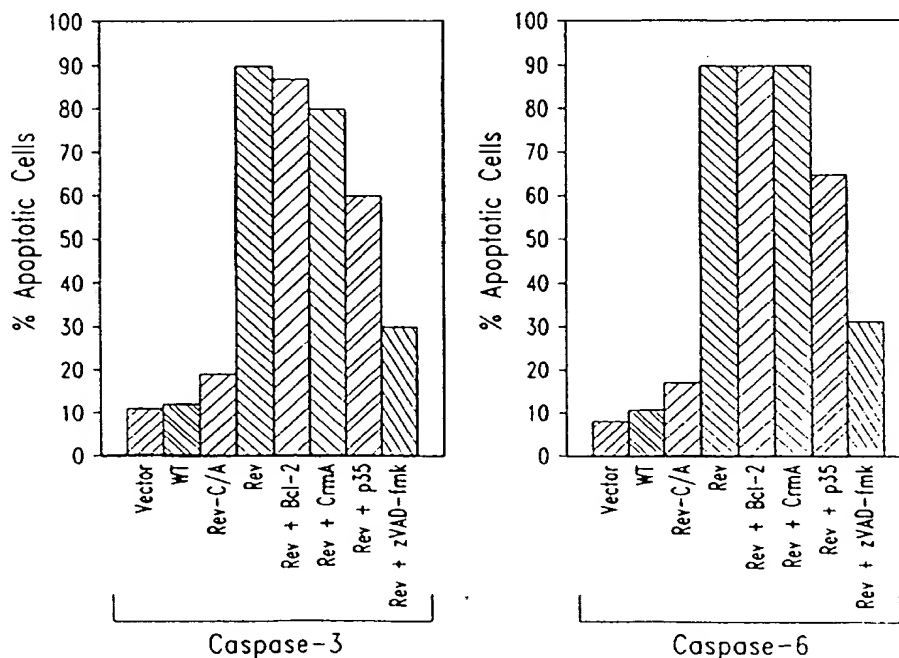
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(54) Title: RECOMBINANT, ACTIVE CASPASES AND USES THEREOF



(57) Abstract

Rev-caspases comprising a primary product in which the small subunit is N-terminal to the large subunit are provided. Rev-caspases are used for screening and identifying caspase inhibitors and enhancers. Rev-caspase genes can be delivered to cells for gene therapy.

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RECOMBINANT, ACTIVE CASPASES AND USES THEREOF

TECHNICAL FIELD

The present invention relates generally to regulating apoptosis, and more particularly to the novel aspartate-specific cysteine proteases known as caspases, their coding regions, mutant forms thereof, and their use in screening assays and as pharmaceutical compositions for the controlled death of targeted cells to treat human disease.

BACKGROUND OF THE INVENTION

Tissue homeostasis is maintained by the process of apoptosis—that is, the normal physiological process of programmed cell death. Changes to the apoptotic pathway that prevent or delay normal cell turnover can be just as important in the pathogenesis of diseases as are abnormalities in the regulation of the cell cycle. Like cell division, which is controlled through complex interactions between cell cycle regulatory proteins, apoptosis is similarly regulated under normal circumstances by the interaction of gene products that either prevent or induce cell death.

Since apoptosis functions in maintaining tissue homeostasis in a range of physiological processes such as embryonic development, immune cell regulation and normal cellular turnover, the dysfunction or loss of regulated apoptosis can lead to a variety of pathological disease states. For example, the loss of apoptosis can lead to the pathological accumulation of self-reactive lymphocytes that occurs with many autoimmune diseases. Inappropriate loss or inhibition of apoptosis can also lead to the accumulation of virally infected cells and of hyperproliferative cells such as neoplastic or tumor cells. Similarly, the inappropriate activation of apoptosis can also contribute to a variety of pathological disease states including, for example, acquired immunodeficiency syndrome (AIDS), neurodegenerative diseases and ischemic injury. Treatments which are specifically designed to modulate the apoptotic pathways in these and other pathological conditions can alter the natural progression of many of these diseases.

Although apoptosis is mediated by diverse signals and complex interactions of cellular gene products, the results of these interactions ultimately feed into a cell death pathway that is evolutionarily conserved between humans and invertebrates. The pathway, itself, is a cascade of proteolytic events analogous to that of the blood coagulation cascade.

Several gene families and products that modulate the apoptotic process have now been identified. One family is the aspartate-specific cysteine proteases ("caspases"). The caspase Ced-3, identified in *C. elegans*, is required for programmed cell death during development of the roundworm *C. elegans*. Ced-3 homologues as well as other caspases have been characterized. The human caspase family includes, for example, human ICE (interleukin-1- β converting enzyme) (caspase-1), ICE_{rel}II (caspase-4), ICE_{rel}III (caspase-5), Mch5 (caspase-8), Mch4 (caspase-10), ICE-LAP6 (caspase-9), Mch2 (caspase-6), CPP32 (caspase-3), ICE-LAP3 (caspase-7), ICH-1 (caspase-2), Caspase 11-14, and others.

The caspases share many features. In this regard, caspases are cysteine proteases (named for a cysteine residue in the active site) that cleave substrates at Asp-X bonds. Furthermore, the primary caspase product is a zymogen that requires proteolytic cleavage at specific internal aspartate residues for activation. The primary gene product is arranged such that the N-terminal peptide (prodomain) precedes a large subunit domain, which precedes a small subunit domain. Cleavage of a caspase yields two subunits, a large (generally approximately 20 kD) and a small (generally approximately 10 kD) subunit that associate non-covalently to form a heterodimer, and, in some caspases, an N-terminal peptide of varying length (see Figure 1). The heterodimer may combine non-covalently to form a tetramer.

Caspase zymogens are themselves substrates for caspases. Inspection of the interdomain linkages in each zymogen reveals target sites (i.e. protease sites) that indicate a hierarchical relationship of caspase activation. By analyzing such pathways, it has been demonstrated that caspases are required for apoptosis to occur. Moreover, caspases appear to be necessary for the accurate and limited proteolytic events which are the hallmark of classic apoptosis (see Salvesen and Dixit, *Cell*,

91:443-446, 1997). However, when overexpressed in mammalian cells, the short prodomain caspases-3 and -6 cells are unable to undergo autocatalytic processing/activation and do not induce apoptosis. Thus, no cellular model system has been developed in which to test inhibitors of these caspases nor is gene delivery
5 of a caspase commonplace.

Therefore, there exists a need in the art for methods of assaying compounds for their ability to affect caspase activity as well as for methods of regulating caspases in order to treat diseases and syndromes. The present invention provides recombinant caspase constructs that are active in cells, allowing the
10 regulation of apoptosis for the treatment of pathology as well as providing methods and compositions for assaying compounds for caspase inhibitory and, thus, anti-apoptotic effects, while further providing other related advantages.

SUMMARY OF THE INVENTION

The present invention generally provides rev-caspases. In one aspect,
15 the invention provides an isolated nucleic acid molecule encoding a rev-caspase. In certain embodiments, the rev-caspase is selected from the group consisting of rev-caspase-1, rev-caspase-2, rev-caspase-3, rev-caspase-4, rev-caspase-5, rev-caspase-6, rev-caspase-7, rev-caspase-8, rev-caspase-9, rev-caspase-10, rev-caspase-11, rev-caspase-12, rev-caspase-13, and rev-caspase-14. In other preferred embodiments, the
20 rev-caspase is a human rev-caspase. Nucleic acid and amino acid sequences of rev-caspases are provided. The invention also provides rev-caspase proteins.

In another aspect, an expression vector comprising the nucleic acid molecule encoding rev-caspase is provided, wherein the sequence encoding rev-caspase is operatively linked to a promoter. In certain embodiments, the promoter is
25 inducible, such as HIV LTR. Host cells transfected with the expression vectors are also provided.

In the present invention, methods of identifying an inhibitor or enhancer of caspase processing activity are provided, comprising: (a) contacting a sample containing an *in vitro* translated rev-caspase with a candidate inhibitor or

candidate enhancer; and (b) detecting the presence of large and small subunits of rev-caspase, and therefrom determining the level of caspase processing activity, wherein a decrease in processing indicates the presence of a caspase inhibitor, and wherein an increase in processing indicates the presence of a caspase enhancer, wherein
5 processed rev-caspase yields large and small subunits.

In other aspects, methods are provided for identifying an inhibitor or enhancer of caspase processing activity, comprising: (a) contacting a cell transfected with the vector expressing rev-caspase with a candidate inhibitor or candidate enhancer; and (b) detecting the presence of large and small subunits of rev-caspase,
10 and therefrom determining the level of caspase processing activity, wherein a decrease in processing indicates the presence of a caspase inhibitor, and wherein an increase in processing indicates the presence of a caspase enhancer, wherein processed rev-caspase yields large and small subunits.

Methods are also provided for identifying an inhibitor or enhancer of
15 caspase-mediated apoptosis, comprising: (a) contacting a cell transfected with the vector expressing rev-caspase with a candidate inhibitor or candidate enhancer or with a reference compound; and (b) detecting cell viability, wherein viability of cells contacted with a candidate is increased in the presence of an inhibitor and is decreased in the presence of an enhancer compared to cells contacted with a reference
20 compound.

In other aspects, gene delivery vehicles, comprising the nucleic acid molecule encoding a rev-caspase are provided, wherein the rev-caspase sequence is operatively linked to a promoter. In preferred embodiments, the gene delivery vehicle is a retrovirus or adenovirus or the nucleic acid molecule is associated with a
25 polycation. The gene delivery vehicle may further comprise a ligand that binds a cell surface receptor.

The invention also provides methods of treating cancer or autoimmune diseases, comprising administering to a patient the gene delivery vehicles disclosed herein.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, the various references set forth below that describe in more detail certain procedures or compositions (e.g., plasmids, etc.), and are therefore incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A is a schematic representation of the processing and folding of human caspase-3 into the mature zymogen represented by bar and ribbon diagrams and is representative of other caspases. α helices are shown as spirals and the β strands are represented by arrows. The N- and the C-termini of the LS are labeled N-LS and C-LS, respectively, and the termini of the SS are similarly labeled N-SS and C-SS.

Figures 1B and C are schematic representations of rev-caspase-3 and -6, respectively. In both Figures 1B and C the N-terminus of the SS and the C-terminus of the LS are labeled as in Figure 1A and the linker region between the C-SS and the N-LS which includes the caspase-3 or -6 prodomain is represented by a thin line. Solid arrows indicate the cleavage sites (DEV Δ G, Asp9 and Asp28—for rev-caspase-3 and VEIDA and Asp23—for rev-caspase-6) within the linker region. The hatched boxes represent a 15 residue-long T7-tag on the N-termini of the wild-type and the rev-caspases. All aspartate processing sites are indicated on the bar diagrams. Figure 1B further depicts a schematic representation of the spontaneous folding of rev-caspase-3 into the mature zymogen represented a ribbon diagram. The ribbon diagram of rev-caspase-3 is based on the published crystal structure of caspase-3.

Figure 2A is a scanned image of an autoradiogram representing SDS-PAGE analysis of rev-caspase autoprocessing. Caspase-3 and -6 or their rev-versions (Rev) including active site Cys to Ala mutants (Rev C'A) in pRSC-lacZ constructs were *in vitro* translated in the presence of 35 S-methionine. The translation products

were then analyzed by SDS-PAGE and autoradiography. The LS and the SS are indicated.

Figures 2B and C are scanned images of autoradiograms representing SDS-PAGE analysis of the autoprocessing of rev-caspase-3 and -6, respectively in the presence of varying levels of selected caspase inhibitors. Rev-caspase-3 (Figure 2B) or rev-caspase-6 (Figure 2C) were *in vitro* translated in the presence of increasing concentrations of DEVD-CHO (0.04 μ M) or zVAD-fmk (0-5 μ M). The translation products were then analyzed as in Figure 2A. WT, wild-type.

Figures 3A and B are scanned images representing the SDS-PAGE analysis of the ability of rev-caspase-3 (imaged by western blot) and -6 (imaged by autoradiogram) to cleave PARP and lamin, respectively. In Figure 3A purified human PARP was incubated with buffer (lane 1) or BL-21 bacterial extracts prepared from bacteria transformed with caspase-3 (lane 2), rev-caspase-3 (lane 3), caspase-6 (lane 4), rev-caspase-6 (lane 5) constructs or empty pET28a vector (lane 6) for 2 h at 37°C. The reaction products were then analyzed by SDS-PAGE and Western blotting with anti-human PARP antibody. In Figure 3B a cDNA encoding the C-terminus of lamin A (amino acids) which contain the caspase-6 cleavage site (VEIDA) was amplified by PCR and *in vitro* translated in the presence of 35 S-methionine. The labeled product was incubated with buffer (lane 1) or the BL-21 bacterial extracts listed above for 2 h at 37°C, and then analyzed by SDS-PAGE and autoradiography. The cleavage products are indicated to the right.

Figures 4A and B are bar diagrams representing the ability of rev-caspase-3 and -6 to induce apoptosis in MCF-7 cells. MCF-7 cells were transiently transfected with either rev-caspase-3 (Figure 4A), or rev-caspase-6 (Figure 4B) expression constructs in combination with 4-fold of CrmA, p35 or Bcl-2 expression constructs, or 20 μ M zVAD-fmk. Cells transfected with an empty vector or the wild-type caspase-3 or -6 were used as controls.

Figure 5A is a scanned image of an autoradiogram representing SDS-PAGE analysis of the enzymatic activity of uncleavable rev-caspase-3. Uncleavable rev-caspase-3 was *in vitro* translated in the absence or the presence of increasing

concentrations of DEVD-CHO. The translation product contains a cleavable 35 residues-long His6-T7-tag at its N-terminus. The active site mutant rev-caspase-3 (Rev C/A) was used as a control. The p32 cleavage product without the His6-T7-tag is indicated to the right.

5 Figure 5B is a plot of an activity assay of bacterially expressed uncleavable rev-caspase-3. The plot measures the ability of the uncleavable rev-caspase-3 to cleave the DEVD-AMC substrate. Rev, rev-caspase-3; Rev-mod, uncleavable rev-caspase-3; Rev-C/A, rev-caspase-3 with an active site mutation.

10 Figure 6 is a multiple amino acid sequence alignment of the relatively conserved regions of the caspases (SEQ ID NOs: 54-115). In the bottom line, "c" refers to residues involved in catalysis, "b" refers to residues that bind the substrate-carboxylate of P1 Asp, "a" refers to residues adjacent to the substrate P2-P4 recognition responsible amino acids, "DX" indicates known and potential processing sites between the small and large subunits of the caspases. The roman numerals at the
15 left of the figure indicate the caspase subfamilies: Ced-like (I), ICE-like (II), and the Nedd2/Ich-1-like (III). The asterisk represents the non-conservative substitution in the active site pentapeptide sequences of Mch4 (caspase-10), Mch5 (caspase-8), and Mch6 (caspase-9)

20 Figure 7 depicts a nucleotide sequence of Rev-caspase-3 (SEQ ID NO:1).

 Figure 8 depicts a nucleotide sequence of uncleavable Rev-caspase-3 (SEQ ID NO:2).

 Figure 9 depicts a nucleotide sequence of Rev-caspase-6 (SEQ ID NO:3).

25 Figure 10 depicts a schematic of some possible rev-caspases. I, intervening sequence; SS, small subunit; P, prodomain; LS, large subunit; X, linker.

 Figures 11A and 11B depict a nucleotide (SEQ ID NOs: 4 and 5) and predicted amino acid sequence of caspase-1 (SEQ ID NO:6).

30 Figures 12A and 12 B depict a nucleotide (SEQ ID NOs: 7 and 8) and predicted amino acid sequence of caspase-2 (SEQ ID NO:9).

Figures 13A and 13B depict a nucleotide (SEQ ID NOs: 10 and 11) and predicted amino acid sequence of caspase-3 (SEQ ID NO:12).

Figures 14A and 14B depict a nucleotide (SEQ ID NOs: 13 and 14) and predicted amino acid sequence of caspase-4 (SEQ ID NO:15).

5 Figures 15A and 15B depict a nucleotide (SEQ ID NOs: 16 and 17) and predicted amino acid sequence of caspase-5 (SEQ ID NO:18).

Figures 16A and 16B depict a nucleotide (SEQ ID NOs: 19 and 20) and predicted amino acid sequence of caspase-6 (SEQ ID NO:21).

10 Figures 17A and 17B depict a nucleotide (SEQ ID NOs: 22 and 23) and predicted amino acid sequence of caspase-7 (SEQ ID NO:24).

Figures 18A-18C depict a nucleotide (SEQ ID NOs: 25 and 26) and predicted amino acid sequence of caspase-8 (SEQ ID NO:27).

Figures 19A and 19B depict a nucleotide (SEQ ID NOs: 28 and 29) and predicted amino acid sequence of caspase-9 (SEQ ID NO: 30).

15 Figures 20A and 20B depict a nucleotide (SEQ ID NOs: 31 and 32) and predicted amino acid sequence of caspase-10 (SEQ ID NO:33).

Figures 21A, 21B, and 21C depict predicted amino acid sequences of Rev-caspase-3 (A: SEQ ID NO:34), uncleavable rev-caspase-3 (B: SEQ ID NO:35), and rev-caspase-6 (C: SEQ ID NO:36).

20 DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention, it may be helpful to an understanding thereof to set forth definitions of certain terms that will be used hereinafter.

As used herein, a caspase refers to a cysteine protease that specifically
25 cleaves proteins after Asp residues. Caspases are initially expressed as zymogens, in which a large subunit is N-terminal to a small subunit. Caspases are generally activated by cleavage at internal Asp residues (Figure 1A). These proteins have been identified in many eukaryotes, including *C. elegans*, *Drosophila*, mouse, and humans. Currently, there are at least 14 known caspase genes, named caspase-1 through
30 caspase-14. Caspases are found in myriad organisms, including human, mouse,

insect (e.g., *Drosophila*), and other invertebrates (e.g., *C. elegans*). In Table 1, ten human caspases are listed along with their alternative names. The nucleotide and amino acid sequences of representative human caspase gene products are presented in SEQ ID NOs: 4-33 and Figures 11-20.

5

Caspase	Alternative name
Caspase-1	ICE
Caspase-2	ICH-1
Caspase-3	CPP32, Yama, apopain
Caspase-4	ICE _{rel} II; TX, ICH-2
Caspase-5	ICE _{rel} III; TY
Caspase-6	Mch2
Caspase-7	Mch3, ICE-LAP3, CMH-1
Caspase-8	FLICE; MACH; Mch5
Caspase-9	ICE-LAP6; Mch6
Caspase-10	Mch4, FLICE-2

As used herein, "rev-caspase" refers to a cysteine protease that specifically cleaves proteins after Asp residues and is expressed as a zymogen, in which a small subunit is N-terminal to a large subunit.

10

Within the context of this invention, it should be understood that a caspase or rev-caspase includes wild-type protein sequences, as well as other variants (including alleles) of the native protein sequence. Briefly, such variants may result from natural polymorphisms or may be synthesized by recombinant methodology, and differ from wild-type protein by one or more amino acid substitutions, insertions, deletions, or the like. Typically, when engineered, amino acid substitutions will be conservative, *i.e.*, substitution of amino acids within groups of polar, non-polar, aromatic, charged, etc. amino acids. In the region of homology to the native sequence, variants should preferably have at least 90% amino acid sequence identity, and within certain embodiments, greater than 92%, 95%, or 97% identity.

15

As will be appreciated by those skilled in the art, a nucleotide sequence encoding a caspase, rev-caspase or variant may differ from the known native sequences, due to codon degeneracies, nucleotide polymorphisms, or amino

20

acid differences. In other embodiments, variants should preferably hybridize to the native nucleotide sequence at conditions of normal stringency, which is approximately 25-30°C below T_m of the native duplex (e.g., 5X SSPE, 0.5% SDS, 5X Denhardt's solution, 50% formamide, at 42°C or equivalent conditions; see generally, Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Press, 1987; Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing, 1987). Low stringency hybridizations utilize conditions approximately 40°C below T_m , and high stringency hybridizations utilize conditions approximately 10°C below T_m . Variants preferably have at least 75% nucleotide identity to native sequence, preferably at least 80%, 85%, and most preferably at least 90% nucleotide identity.

An "isolated nucleic acid molecule" refers to a polynucleotide molecule in the form of a separate fragment or as a component of a larger nucleic acid construct, that has been separated from its source cell (including the chromosome it normally resides in) at least once in a substantially pure form. Nucleic acid molecules may be comprised of a wide variety of nucleotides, including DNA, RNA, nucleotide analogues, or some combination of these.

A. CASPASE AND REV-CASPASE GENES AND GENE PRODUCTS

As noted above, the invention provides compositions relating to caspase and rev-caspase genes and gene products, and methods for the use of the genes and gene products. In particular, the invention provides rev-caspase constructs that are active when expressed in cells. Given the disclosure provided herein, a caspase gene can be isolated from a variety of cell types and engineered to produce a rev-caspase.

1. Isolation of caspase genes

The present invention, as described herein, provides rev-caspase genes, which are constructed from caspase genes. Caspase genes may be isolated from either genomic DNA or preferably cDNA. Isolation of caspase genes from genomic DNA or cDNA typically can proceed by, first, generating an appropriate DNA library

through techniques for constructing libraries that are known in the art (*see* Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, 1989) or purchased from commercial sources (*e.g.*, Clontech, Palo Alto, CA). Briefly, cDNA libraries can be constructed in bacteriophage vectors (*e.g.*, λ ZAPII), plasmids, or others, which are suitable for screening, while genomic DNA libraries can be constructed in chromosomal vectors, such as YAC's (yeast artificial chromosomes), bacteriophage vectors, such as λ EMBL3, λ gt10, cosmids, or plasmids.

In one embodiment known caspase sequences may be utilized to design an oligonucleotide hybridization probe suitable for screening genomic or cDNA libraries. Preferably, such oligonucleotide probes are 20-30 bases in length. To facilitate hybridization detection, the oligonucleotide may be conveniently labeled, generally at the 5' end, with a reporter molecule, such as a radionuclide, (*e.g.*, ^{32}P), enzymatic label, protein label, fluorescent label, or biotin. Such libraries are then generally plated as phage or colonies, depending upon the vector used. Subsequently, a nitrocellulose or nylon membrane, to which the colonies or phage have been transferred, is probed to identify candidate clones which contain the caspase gene. Such candidates may be verified as containing caspase DNA by any of various means including, for example, DNA sequence analysis or hybridization with a second, non-overlapping probe.

Once a library is identified as containing a caspase gene, the gene can be isolated by amplification. Briefly, when using cDNA library DNA as a template amplification primers are designed based upon known caspase gene sequences (*see* GenBank Accession Nos. X65019 (caspase-1), U13021 (caspase-2), U13737 (caspase-3), U25804 (caspase-4), U28015 (caspase-5), U20536 (caspase-6), U37448 (caspase-7), U60520 (caspase-8), U56390 (caspase-9), U60519 (caspase-10), Y13089 (caspase-11), Y13090 (caspase-12), AF078533 (caspase-13), AF092997 (caspase-14), and sequences provided herein). Amplification of cDNA libraries made from cells with high caspase activity is preferred. Primers for amplification are preferably derived from sequences in the 5' and 3' untranslated region in order to isolate a full-length cDNA. The primers preferably have a GC content of about 50% and contain

restriction sites to facilitate cloning and do not have self-complementary sequences nor do they contain complementary sequences at their 3' end (to prevent primer-dimer formation). The primers are annealed to cDNA or genomic DNA and sufficient amplification cycles are performed to yield a product readily visualized by gel electrophoresis and staining. The amplified fragment is purified and inserted into a vector, such as λ gt10 or pBS(M13+), and propagated. Confirmation of the nature of the fragment is obtained by DNA sequence analysis or indirectly through amino acid sequencing of the encoded protein.

Other methods may also be used to obtain a caspase encoding nucleic acid molecule. For example, a nucleic acid molecule encoding caspase may be obtained from an expression library by screening with an antibody or antibodies reactive to caspase (see, Sambrook, et al. *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, NY, 1987; Ausubel, et al. *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley-Interscience, NY, 1995).

Caspase genes from a variety of species may be isolated using the compositions provided herein. For closely related species, the human sequence or portion thereof may be utilized as a probe on a genomic or cDNA library. For example, a fragment of caspase that encompasses the catalytic site may be labeled and used as a probe on a library constructed from mouse, primate, rat, dog, or other vertebrate, warm-blooded or mammalian species. An initial hybridization at normal stringency may yield candidate clones or fragments. If no hybridization is initially observed, varying degrees of stringency may be used. (see Sambrook et al. *supra*, and other well-known sources for stringency conditions) While such probes may also be used to probe libraries from evolutionarily diverse species, such as *Drosophila*, hybridization conditions will likely be more relaxed.

While relaxed hybridization conditions using probes designed from human sequences may identify caspase genes of evolutionarily diverse species it may be more beneficial to attempt to directly isolate these genes from a library using methods which do not require the human sequence per se. These methods include,

but are not limited to, amplification using primers derived from conserved areas, amplification using degenerate primers from various regions, antibody probing of expression libraries, and the like. For example, random-primed amplification (e.g., polymerase chain reaction) may be employed (*see, e.g., Methods Enzymol.* 254: 275, 5 1995; *Trends Genet.* 11: 242, 1995; Liang and Pardee, *Science* 257: 967, 1992; Welsh et al., *Nucl. Acids Res.* 20: 4965, 1992). In addition, variations of random-primed PCR may also be used, especially when a particular gene or gene family is desired. In such a method, one of the amplification primers is an "anchored oligo(dT) (oligo(dT)dN)" and the other primer is a degenerate primer based upon amino acid or 10 nucleotide sequence of a related gene. A gene sequence is identified as a caspase by amino acid similarity and / or nucleic acid similarity. Generally, amino acid similarity is preferred. Candidate caspase genes are examined for enzyme activity by one of the functional assays described herein or other equivalent assays.

Variants of caspase and rev-caspase genes provided herein may be 15 engineered from natural variants (e.g., polymorphisms, splice variants, mutants), synthesized or constructed. Many methods have been developed for generating mutants (*see, generally, Sambrook et al., supra; Ausubel, et al., supra, and the discussion above*). Briefly, preferred methods for generating a few nucleotide substitutions utilize an oligonucleotide that spans the base or bases to be mutated and 20 contains the mutated base or bases. The oligonucleotide is hybridized to complementary single stranded nucleic acid and second strand synthesis is primed from the oligonucleotide. The double-stranded nucleic acid is prepared for transformation into host cells, typically *E. coli*, but alternatively, other prokaryotes, yeast or other eukaryotes. Standard screening and vector growth protocols are used to 25 identify mutant sequences and obtain high yields.

Similarly, deletions and/or insertions of the caspase or rev-caspase genes may be constructed by any of a variety of known methods as discussed *supra*. For example, the gene can be digested with restriction enzymes and religated such that a sequence is deleted or religated with additional sequences such that an insertion 30 or large substitution is made. Other means of generating variant sequences may be

employed with methods known in the art, for example those described in Sambrook et al. (*supra*) and Ausubel et al. (*supra*). Verification of variant sequences is typically accomplished by restriction enzyme mapping, sequence analysis, or probe hybridization. Variants which catalyze Asp-specific cleavages are useful in the
 5 context of this invention.

B. REV-CASPASES

The caspases of the present invention are generated by rearranging the gene sequence of the caspase gene such that the nucleic acid sequence encoding the small subunit precedes (is 5' to) the nucleic acid sequence encoding the large subunit.
 10 These rearranged caspases are called rev-caspases.

1. Structure of rev-caspases

The rev-caspases of the present invention comprise at least a portion of the small subunit and at least a portion of the large subunit. In preferred embodiments, the prodomain or a portion thereof (see Figures 1, 10) and/or an
 15 intervening sequence or a portion thereof (see Figure 10) are also present in rev-caspase. In other preferred embodiments, a "linker" region is located between the small and large subunits.

The boundaries of the small subunit and large subunit are identified either experimentally by amino acid sequence analysis of the mature caspase or by
 20 inspection of structural homology (*e.g.*, the conserved Asp-X cleavage site). For exemplary purposes, the Table below presents the boundaries of the prodomain (P), large subunit (LS), intervening sequence (I), and small subunit (SS) of human caspase-1 through -10. The nucleotide numbers refer to the nucleotides in SEQ ID NOs: 4-33 and in Figures 11-20.

25

Caspase	Prodomain	Large Subunit	Intervening sequence	Small Subunit
Caspase-1	1-357	358-891	892-948	949-1212
Caspase-2	1-456	457-948	949-990	991-1305
Caspase-3	1-84	85-525		526-831

Caspase-4	1-240	241-810	811-867	868-1131
Caspase-5	1-363	364-933		934-1254
Caspase-6	1-69	70-537	538-579	580-879
Caspase-7	1-69	70-594		595-909
Caspase-8	1-681	682-1173		1174-1488
Caspase-9	1-390	391-945	946-990	991-1248
Caspase-10	1-657	658-1116		1117-1437

As noted above, a portion of the large subunit and small subunit may be used in rev-caspase constructs. When designing rev-caspases that contain a portion of these subunits, the active site (*e.g.*, QACXG, where X is Arg, Gln, or Gly), which is located near the C-terminus of the large subunit should not be deleted if protease activity is desired. Preferably, the 3-dimensional structure as determined by X-ray crystallography (see Mittl et al., *J. Biol. Chem.*, 272:6539-6547, 1997; Rotonde et al., *Nat. Struct. Biol.*, 3:619-625, 1996; Walker et al., *Cell*, 78:343-352, 1994; Wilson et al., *Nature*, 370:270-275, 1994) is maintained. For example, from the x-ray crystallographic structures of caspases, the amino acids, that are important in binding substrates have been identified. Likewise, substitutions of amino acids in the active site may be detrimental to maintaining activity. Although it is preferred that both subunits are derived from the same caspase, combinations of subunits from different caspases and/or from different species may be used.

The prodomain (sometimes called an N-terminal peptide) is generally not required for enzyme activity and is normally released *in vivo*. Rev-caspases of the present invention optionally have a prodomain or portion thereof. Similarly, the intervening sequence, which is present in certain caspases, is optional for inclusion in rev-caspases.

In certain embodiments, a linker region is engineered between the small and large subunits. A "linker region", as used herein, refers to a peptide of from about 5 to about 50 amino acids. In preferred embodiments, the linker may contain a protease sensitive or cleavage site. Any site recognized by an intracellular protease may be used. In addition, multiple protease sensitive sites may be tandemly

arranged in the same linker. Preferred protease sensitive sites are susceptible to cleavage by caspases or by viral proteases. Preferred caspase sensitive sites include, but are not limited to DXXDG (wherein X is any amino acid; SEQ ID NO:37); DEV DG (SEQ ID NO:38), IETDG (SEQ ID NO:39), YVADG (SEQ ID NO:40),
5 YVHDG (SEQ ID NO:41), and WEHDG (SEQ ID NO:42). Furthermore, the Gly residue may be Ala or another small amino acid. The latter three sites are specifically cleaved by caspases-1, -4, and -5. Other sites specifically cleaved by only one or a few caspases are preferred in certain embodiments. Viral proteases cleavage sites include, but are not limited to, those recognized and cleaved by HIV protease, HCV
10 (hepatitis C virus) protease, HBV (hepatitis B virus) protease, and rhinovirus protease.

2. *Construction of rev-caspases*

Rev-caspases may be constructed from caspase sequences by a variety of methods known in the art. A preferred method is amplification (*e.g.*, polymerase
15 chain reaction (PCR)) to selectively amplify the individual subunits and place these in cloning vectors such as pUC such as described in Example 1. Moreover, such PCR reactions can be performed in a variety of ways such that the primers used for amplification contain specific restriction endonuclease sites to facilitate insertion into a vector.

20 Further, a variety of other methodologies besides PCR may be used to attain the desired rearrangement. For example, one skilled in the art may employ isothermal methods to amplify the nucleotide sequence of interest, using existing restriction endonuclease sites present in the nucleotide sequence to excise and insert sequences, or by the introduction of distinct restriction endonuclease sites by site-
25 directed mutagenesis followed by excision and insertion. These and other methods are described in Sambrook et al., *supra*; Ausubel, et al., *supra*. Briefly, one methodology is to generate single-stranded cDNA of the caspase of interest, followed by annealing a primer, which is complementary except for the desired alteration (*e.g.*, a small insertion, deletion, or mutation such that a unique restriction site is created
30 between the large and small subunits and/or at the 5' and 3' ends of both subunits).

Bacterial cells are transformed and screened for those which contain desired construct. This construct is then digested to liberate the subunit sequences, which can then be purified and religated into the appropriate orientation.

As indicated above, rev-caspase genes may be manipulated to contain
5 insertions, deletions or substitutions. Moreover, such variant rev-caspase genes useful in the context of this invention include those which facilitate Asp-specific cleavages indicative of caspase activity. Further, variants which are incapable of being cleaved into separate subunits are encompassed within the context of this invention, if those variants are able to facilitate Asp-specific cleavages by way of a
10 cysteine-containing active site. By way of guidance, amino acids involved in catalysis, Asp recognition in substrate, and P2-P4 substrate recognition are provided in Figure 6.

C. VECTORS, HOST CELLS AND MEANS OF EXPRESSING AND PRODUCING PROTEIN

Caspase may be expressed in a variety of host organisms. In certain
15 embodiments, caspase is produced in bacteria, such as *E. coli*, or mammalian cells (e.g., CHO and COS-7), for which many expression vectors have been developed and are available. Other suitable host organisms include other bacterial species, and eukaryotes, such as yeast (e.g., *Saccharomyces cerevisiae*), and insect cells (e.g., Sf9).

20 A DNA sequence encoding rev-caspase is introduced into an expression vector appropriate for the host. In certain embodiments, rev-caspase is inserted into a vector such that a fusion protein is produced. The rev-caspase sequence is derived as described herein. As discussed above, the sequence may contain alternative codons for each amino acid with multiple codons. The alternative
25 codons can be chosen as "optimal" for the host species. Restriction sites are typically incorporated into the primer sequences and are chosen with regard to the cloning site of the vector. If necessary, translational initiation and termination codons can be engineered into the primer sequences.

At minimum, the vector must contain a promoter sequence. As used herein, a "promoter" refers to a nucleotide sequence that contains elements that direct the transcription of a linked gene. At minimum, a promoter contains an RNA polymerase binding site. More typically, in eukaryotes, promoter sequences contain
5 binding sites for other transcriptional factors that control the rate and timing of gene expression. Such sites include TATA box, CAAT box, POU box, AP1 binding site, and the like. Promoter regions may also contain enhancer elements. When a promoter is linked to a gene so as to enable transcription of the gene, it is "operatively linked".

10 Other regulatory sequences may be included. Such sequences include a transcription termination signal sequence, secretion signal sequence, origin of replication, selectable marker, and the like. The regulatory sequences are operationally associated with one another to allow transcription or translation.

The expression vectors used herein include a promoter designed for
15 expression of the proteins in a host cell (*e.g.*, bacterial). Suitable promoters are widely available and are well known in the art. Inducible or constitutive promoters are preferred. Such promoters for expression in bacteria include promoters from the T7 phage and other phages, such as T3, T5, and SP6, and the *trp*, *lpp*, and *lac* operons. Hybrid promoters (*see*, U.S. Patent No. 4,551,433), such as *tac* and *trc*, may
20 also be used. Promoters for expression in eukaryotic cells include the P10 or polyhedron gene promoter of baculovirus/insect cell expression systems (*see, e.g.*, U.S. Patent Nos. 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784), MMTV LTR, CMV IE promoter, RSV LTR, SV40, metallothionein promoter (*see, e.g.*, U.S. Patent No. 4,870,009) and the like.

25 The promoter controlling transcription of rev-caspase may itself be controlled by a repressor. In some systems, the promoter can be derepressed by altering the physiological conditions of the cell, for example, by the addition of a molecule that competitively binds the repressor, or by altering the temperature of the growth media. Preferred repressor proteins include, but are not limited to the *E. coli*

lacI repressor responsive to IPTG induction, the temperature sensitive λ cl857 repressor, and the like. The *E. coli* lacI repressor is preferred.

In other preferred embodiments, the vector also includes a transcription terminator sequence. A "transcription terminator region" has either a
5 sequence that provides a signal that terminates transcription by the polymerase that recognizes the selected promoter and/or a signal sequence for polyadenylation.

Preferably, the vector is capable of replication in the host cells. Thus, when the host cell is a bacterium, the vector preferably contains a bacterial origin of replication. Preferred bacterial origins of replication include the fl-ori and col E1
10 origins of replication, especially the ori derived from pUC plasmids. In yeast, ARS or CEN sequences can be used to assure replication. A well-used system in mammalian cells is SV40 ori.

The plasmids also preferably include at least one selectable marker that is functional in the host. A selectable marker gene includes any gene that confers
15 a phenotype on the host that allows transformed cells to be identified and selectively grown. Suitable selectable marker genes for bacterial hosts include the ampicillin resistance gene (Amp^r), tetracycline resistance gene (Tet^r) and the kanamycin resistance gene (Kan^r). The kanamycin resistance gene is presently preferred. Suitable markers for eukaryotes usually require a complementary deficiency in the
20 host (e.g., thymidine kinase (tk) in tk- hosts). However, drug markers are also available (e.g., G418 resistance and hygromycin resistance).

The sequence of nucleotides encoding rev-caspase may also include a secretion signal, whereby the resulting peptide is a precursor protein processed and secreted. The resulting processed protein may be recovered from the periplasmic
25 space or the fermentation medium. Secretion signals suitable for use are widely available and are well known in the art (von Heijne, *J. Mol. Biol.* 184:99-105, 1985). Prokaryotic and eukaryotic secretion signals that are functional in *E. coli* (or other host) may be employed. The presently preferred secretion signals include, but are not limited to, those encoded by the following *E. coli* genes: pelB (Lei et al., *J.*

Bacteriol. 169:4379, 1987), *phoA*, *ompA*, *ompT*, *ompF*, *ompC*, beta-lactamase, and alkaline phosphatase.

One skilled in the art appreciates that there are a wide variety of suitable vectors for expression in bacterial cells and which are readily obtainable. Vectors such as the pET series (Novagen, Madison, WI), the *tac* and *trc* series (Pharmacia, Uppsala, Sweden), pTTQ18 (Amersham International plc, England), pACYC 177, pGEX series, and the like are suitable for expression of a rev-caspase. Baculovirus vectors, such as pBlueBac (*see, e.g.*, U.S. Patent Nos. 5,278,050, 5,244,805, 5,243,041, 5,242,687, 5,266,317, 4,745,051, and 5,169,784; available from Invitrogen, San Diego) may be used for expression in insect cells, such as *Spodoptera frugiperda* sf9 cells (*see*, U.S. Patent No. 4,745,051). The choice of a bacterial host for the expression of a rev-caspase is dictated in part by the vector. Commercially available vectors are paired with suitable hosts.

A wide variety of suitable vectors for expression in eukaryotic cells are available. Such vectors include pCMVLacI, pXT1 (Stratagene Cloning Systems, La Jolla, CA); pCDNA series, pREP series, pEBVHis (Invitrogen, Carlsbad, CA). In certain embodiments, rev-caspase gene is cloned into a gene targeting vector, such as pMC1neo, a pOG series vector (Stratagene Cloning Systems).

Rev-caspase is isolated by standard methods, such as affinity chromatography, size exclusion chromatography, metal ion chromatography, ionic exchange chromatography, HPLC, and other known protein isolation methods. (*see generally* Ausubel et al. *supra*; Sambrook et al. *supra*). An isolated purified protein gives a single band on SDS-PAGE when stained with Coomassie blue.

Rev-caspase may be expressed as a hexa-his fusion protein and isolated by metal-containing chromatography, such as nickel-coupled beads. Briefly, a sequence encoding His₆ is linked to a DNA sequence encoding a rev-caspase. Although the His₆ sequence can be positioned anywhere in the molecule, preferably it is linked at the 3' end immediately preceding the termination codon. The fusion may be constructed by any of a variety of methods. A convenient method is amplification of the rev-caspase gene using a downstream primer that contains the codons for His₆.

Purified rev-caspase protein may be used in assays to screen for inhibitory drugs. These assays may be performed *in vitro* or *in vivo* and utilize any of the methods described herein or that are known in the art. The protein may also be crystallized and subjected to X-ray analysis to determine its 3-dimensional structure or used to raise antibodies.

D. USES OF REV-CASPASE GENE AND GENE PRODUCT

1. *Inhibitors and enhancers of caspase activity*

Candidate inhibitors and enhancers may be isolated or procured from a variety of sources, such as bacteria, fungi, plants, parasites, libraries of chemicals, peptides or peptide derivatives and the like. Inhibitors and enhancers may be also be rationally designed, based on the protein structure determined from X-ray crystallography (see, Mittl et al., *J. Biol. Chem.*, 272:6539-6547, 1997). In certain preferred embodiments, the inhibitor targets a specific caspase (e.g., caspase-3 and not any other caspases).

Without being held to a particular mechanism, the inhibitor may act by preventing processing of caspase or by preventing enzymatic activity, or by other mechanism. The inhibitor may act directly or indirectly. In preferred embodiments, inhibitors interfere in the processing of the caspase protein. In other preferred embodiments, the inhibitors are small molecules. In a most preferred embodiment, the inhibitors prevent apoptosis. Inhibitors should have a minimum of side effects and are preferably non-toxic. Inhibitors that can penetrate cells are preferred.

In addition, enhancers of caspase activity or expression are desirable in certain circumstances. At times, increasing apoptosis will have a therapeutic effect. For example, tumors or cells that mediate autoimmune diseases are appropriate cells for destruction. Enhancers may increase the rate or efficiency of caspase processing, increase transcription or translation, or act through other mechanisms. As is apparent to one skilled in the art, many of the guidelines presented above apply to the design of enhancers as well.

Screening assays for inhibitors and enhancers will vary according to the type of inhibitor or enhancer and the nature of the activity that is being affected. Assays may be performed *in vitro* or *in vivo*. In general, *in vitro* assays are designed to evaluate caspase protein processing or caspase enzymatic activity, and *in vivo* assays are designed to evaluate caspase protein processing, caspase enzymatic activity, apoptosis, or caspase cleavage of substrate. In any of the assays, a statistically significant increase or decrease compared to a proper control is indicative of enhancement or inhibition.

One *in vitro* assay can be performed by examining the effect of a candidate compound on processing of rev-caspase into two subunits. Briefly, a cleavable form of rev-caspase, that is a primary translation product, is obtained from an *in vitro* translation system. The cleavable form of rev-caspase is preferably constructed to be auto-cleaved, but can be constructed to be cleaved by other protease components present or added to the reaction. This primary product is contacted with or without or translated in the presence or absence of a candidate compound and assessed for appearance of the two subunits. to facilitate detection, typically, the primary product of rev-caspase is labeled during translation, cell viability, and the like. The two subunits may be readily detected by autoradiography after gel electrophoresis. One skilled in the art will recognize that other methods of labeling and detection may be used alternatively.

An alternative *in vitro* assay is designed to measure cleavage of a caspase substrate (*e.g.*, Acetyl DEVD-aminomethyl coumarin (amc), lamin, PRPP, and the like). Substrate turnover may be assayed using either cleavable or noncleavable rev-caspase. Briefly, in this method, rev-caspase is translated and allowed sufficient time to be processed, if a cleavable rev-caspase is being used. The caspase substrate along with the candidate compound is added to the reaction. Detection of cleaved substrate is performed by any one of a variety of standard methods. Generally, the substrate will be labeled and followed by an appropriate detection means.

Moreover, any known enzymatic analysis can be used to follow the inhibitory or enhancing ability of a candidate compound with regard to a rev-caspase of this invention. For example, one could express the rev-caspase of interest in a cell line be it bacterial, insect, mammalian or other, either in cleavable or noncleavable form and purify the rev-caspase. The purified rev-caspase could then be used in a variety of assays to follow its catalytic ability in the presence of candidate compounds, as noted above. Such methods of expressing and purifying recombinant proteins are known in the art and examples can be found in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, 1989 as well as in a number of other sources.

In vivo assays are typically performed in cells transfected either transiently or stably with an expression vector containing a rev-caspase gene, such as those described herein. These cells are used to measure rev-caspase processing, substrate turnover, or apoptosis in the presence or absence of a candidate compound. When assaying apoptosis, a variety of cell analyses may be used including, for example, dye staining and microscopy to examine nucleic acid fragmentation and porosity of the cells. Further, *in vivo* assaying for the ability of the transfected rev-caspase to cleave known substrates that are co-transfected or placed in the cell culture media in the presence of the candidate compound can be performed thereby allowing for the detection and determination of substrate turnover.

The assays briefly described herein may be used to identify an enhance or inhibitor that is specific for an individual caspase. In a preferred embodiment candidate compounds would be analyzed using a variety of rev-caspases (e.g., rev-caspase-1 through rev-caspase-14) to identify specific for individual caspases.

A variety of methodologies exist can be used to investigate the effect of a candidate compound. Such methodologies are those commonly used to analyze enzymatic reactions and include, for example, SDS-PAGE, spectroscopy, HPLC analysis, autoradiography, chemiluminescence, chromogenic reactions, and immunochemistry (e.g., blotting, precipitating, etc.).

Inhibitors and enhancers may be used in the context of this invention to exert control over the cell death process or cytokine activation (e.g., IL-1, which is activated by caspase-1). Thus, these inhibitors and enhancers will have utility in diseases characterized by either excessive or insufficient levels of apoptosis.

5 Inhibitors of proteases have potential to treat the major neurodegenerative diseases: stroke, Parkinson's Disease, Alzheimer's Disease, and ALS. As well, caspase protease inhibitors may be used to inhibit apoptosis in the heart following myocardial infarction, in the kidney following acute ischemia, and in diseases of the liver. In other embodiments, inhibitors of caspase-1 can be used to inhibit the release of the
10 pro-inflammatory IL-1 β , and thus may provide therapeutic benefit in treating inflammation and/or autoimmune disorders. Enhancers of caspase activity may be used in contexts when apoptosis or cytokine activation are desired. For example, inducing or increasing apoptosis in cancer cells or aberrantly proliferating cells may be effected by delivery of a caspase enhancer.

15 The inhibitors and enhancers may be further coupled with a targeting moiety that binds a cell surface receptor specific to the cells. Administration of inhibitors or enhancers will generally follow established protocols. The compounds of the present invention may be administered either alone, or as a pharmaceutical composition. Briefly, pharmaceutical compositions of the present invention may
20 comprise one or more of the inhibitors or enhancers as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like, carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol, proteins, polypeptides or amino
25 acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide) and preservatives. In addition, pharmaceutical compositions of the present invention may also contain one or more additional active ingredients.

Compositions of the present invention may be formulated for the
30 manner of administration indicated, including for example, for oral, nasal, venous,

intracranial, intraperitoneal, subcutaneous, or intramuscular administration. Within other embodiments of the invention, the compositions described herein may be administered as part of a sustained release implant. Within yet other embodiments, compositions of the present invention may be formulized as a lyophilizate, utilizing
5 appropriate excipients which provide stability as a lyophilizate, and subsequent to rehydration.

2. *Gene therapy*

As noted above, rev-caspases may be delivered to cells as part of gene delivery vehicles. In many diseases and syndromes, too little apoptosis is an
10 important feature in their development. Treatment of many autoimmune diseases and tumors would benefit from increased apoptosis. One means to increase apoptosis is to provide target cells with caspase genes in an expressible form. This may be accomplished by delivery of DNA or cDNA capable of *in vivo* transcription of the rev-caspase. More specifically, in order to produce rev-caspases *in vivo*, a nucleic
15 acid sequence coding for the rev-caspase is placed under the control of a eukaryotic promoter (e.g., a pol III promoter, CMV or SV40 promoter). Where it is desired to more specifically control transcription, the rev-caspase may be placed under the control of a tissue or cell specific promoter (e.g., to target cells in the liver), or an inducible promoter, such as metallothionein.

20 Many techniques for introduction of nucleic acids into cells are known. Such methods include retroviral vectors and subsequent retrovirus infection, adenoviral or adeno-associated viral vectors and subsequent infection, and complexes of nucleic acid with a condensing agent (e.g., poly-lysine). These complexes or viral vectors may be targeted to particular cell types by way of a ligand incorporated into
25 the vehicle. Many ligands specific for tumor cells and other cells are well known in the art.

A wide variety of vectors may be utilized within the context of the present invention, including for example, plasmids, viruses, retrotransposons and cosmids. Representative examples include adenoviral vectors (e.g., WO 94/26914,
30 WO 93/9191; Yei et al., *Gene Therapy* 1:192-200, 1994; Kolls et al., *PNAS*

91(1):215-219, 1994; Kass-Eisler et al., *PNAS* 90(24):11498-502, 1993; Guzman et al., *Circulation* 88(6):2838-48, 1993; Guzman et al., *Cir. Res.* 73(6):1202-1207, 1993; Zabner et al., *Cell* 75(2):207-216, 1993; Li et al., *Hum Gene Ther.* 4(4):403-409, 1993; Caillaud et al., *Eur. J. Neurosci.* 5(10):1287-1291, 1993), adeno-associated type 1 ("AAV-1") or adeno-associated type 2 ("AAV-2") vectors (see WO 95/13365; Flotte et al., *PNAS* 90(22):10613-10617, 1993), hepatitis delta vectors, live, attenuated delta viruses and herpes viral vectors (e.g., U.S. Patent No. 5,288,641), as well as vectors which are disclosed within U.S. Patent No. 5,166,320. Other representative vectors include retroviral vectors (e.g., EP 0 415 731; WO 90/07936; WO 91/02805; WO 94/03622; WO 93/25698; WO 93/25234; U.S. Patent No. 5,219,740; WO 93/11230; WO 93/10218).

Within certain aspects of the invention, nucleic acid molecules that encode the rev-caspase may be introduced into a host cell utilizing a vehicle, or by various physical methods. Representative examples of such methods include transformation using calcium phosphate precipitation (Dubensky et al., *PNAS* 81:7529-7533, 1984), direct microinjection of such nucleic acid molecules into intact target cells (Acsadi et al., *Nature* 352:815-818, 1991), and electroporation whereby cells suspended in a conducting solution are subjected to an intense electric field in order to transiently polarize the membrane, allowing entry of the nucleic acid molecules. Other procedures include the use of nucleic acid molecules linked to an inactive adenovirus (Cotton et al., *PNAS* 89:6094, 1990), lipofection (Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413-7417, 1989), microprojectile bombardment (Williams et al., *PNAS* 88:2726-2730, 1991), polycation compounds such as polylysine, receptor specific ligands, liposomes entrapping the nucleic acid molecules, spheroplast fusion whereby *E. coli* containing the nucleic acid molecules are stripped of their outer cell walls and fused to animal cells using polyethylene glycol, viral transduction, (Cline et al., *Pharmac. Ther.* 29:69, 1985; and Friedmann et al., *Science* 244:1275, 1989), and DNA ligand (Wu et al., *J. of Biol. Chem.* 264:16985-16987, 1989), as well as psoralen inactivated viruses such as Sendai or

Adenovirus. In one embodiment, the rev-caspase construct is introduced into the host cell using a liposome.

As noted above, pharmaceutical compositions also are provided by this invention. These compositions may contain any of the above described
5 inhibitors, enhancers, DNA molecules, vectors or host cells, along with a pharmaceutically or physiologically acceptable carrier, excipients or diluents. Generally, such carriers should be nontoxic to recipients at the dosages and concentrations employed. Ordinarily, the preparation of such compositions entails combining the therapeutic agent with buffers, antioxidants such as ascorbic acid, low
10 molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents.

In addition, the pharmaceutical compositions of the present invention
15 may be prepared for administration by a variety of different routes, including for example intraarticularly, intracranially, intradermally, intrahepatically, intramuscularly, intraocularly, intraperitoneally, intrathecally, intravenously, subcutaneously or even directly into a tumor. In addition, pharmaceutical compositions of the present invention may be placed within containers, along with
20 packaging material which provides instructions regarding the use of such pharmaceutical compositions. Generally, such instructions will include a tangible expression describing the reagent concentration, as well as within certain embodiments, relative amounts of excipient ingredients or diluents (*e.g.*, water, saline or PBS) which may be necessary to reconstitute the pharmaceutical composition.
25 Pharmaceutical compositions are useful for both diagnostic or therapeutic purposes.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease. Dosages
30 may be determined most accurately during clinical trials. Patients may be monitored

for therapeutic effectiveness by appropriate technology, including signs of clinical exacerbation, imaging and the like.

The following examples are offered by way of illustration, and not by
5 way of limitation.

EXAMPLES

EXAMPLE 1

GENERATION OF cDNAs EXPRESSING REV-CASPASE-3 AND 6 PRECURSORS

5

Generation of cDNAs encoding rev-caspase-3 and 6 precursors were generated by PCR. The large (LS) and small subunits (SS) of caspase-3 were amplified with the following primers using the caspase-3 cDNA as a template:

LS-forward, ATGGAGAACACTGAAACTCAG (SEQ ID NO:43);
10 LS-reverse, GTCATCATCAACACCTCAGTCT (SEQ ID NO:44);
SS-forward, GGATCCATGATTGAGACAGACAGTGG (SEQ ID NO:45);
SS-reverse, ATCAACTTCATCGTGATAAAAAATAGAGTTC (SEQ ID NO:46).

15 The PCR products were cloned separately into the Sma I site of pBluescript KS⁺. The small subunit was then excised from KS⁺-vector with Bam HI and inserted into the Bam HI site of the second KS⁺-vector which contains the large subunit. This places the small subunit in-frame 5' to the large subunit. Rev-caspase-6 was amplified and cloned in the KS⁺-vector in a similar way. The following PCR
20 primers were used with caspase-6-His6 cDNA as a template:

LS-forward, ATGAGCTCGGCCTCGGGG (SEQ ID NO:47);
LS-reverse, TTAATCTACTACATCCAAAGG (SEQ ID NO:48);
SS-forward,
GGATCCATGGTAGAAATAGATGCAGCCTCCGTTTAC (SEQ ID NO:49)
25 SS-reverse, ATCAATTTC AACGTGGTGGTGGTGGTGGTGC (SEQ ID NO:50).

The resulting nucleotide sequences were such that the wild type subunit order was reversed thus creating a contiguous nucleotide sequence wherein the coding region for the small subunit preceded that of the large subunit (See Figure
30 1). The engineered contiguous caspase-3 and 6 molecules (i.e., rev-caspase molecules) in which the SS was fused in frame N-terminal to the LS, and a cleavage

site (DEVVG in the case of caspase-3; VEIDS in the case of caspase-6 (these internal cleavage sites were designed to be specific for the caspase in which it was introduced in order to investigate the autocatalytic activity of the particular caspase)) was introduced between the two subunits and is depicted in Figures 1B and C.

5 To express the rev-caspases in bacteria, their cDNAs were excised with Bam HI/Xho I and subcloned into the bacterial expression vector pET28a (Novagen, Inc.) in-frame with the T7-tag of this vector.

10

EXAMPLE 2

EXPRESSION OF REV-CASPASES IN MAMMALIAN CELLS AND ASSAY FOR APOPTOSIS

To express the rev-caspases in mammalian cells and assay their apoptotic activity, they were amplified with the T7-tag primer and the LS-reverse
15 primers using the pET28a constructs as templates and subcloned into the mammalian double expression vector pRSC-LacZ (MacFarlane et al., *J. Biol. Chem.*, 272:25417-25420, 1997; Tsang et al., *Bio/Technology*, 22:68, 1997). This vector allows the expression of lacZ under the Rous Sarcoma virus promoter, and the test cDNA under the CMV promoter. To assay for apoptosis, MCF-7 or 293 cells were transfected,
20 using the method commercially available as the Lipofect Amine method (Life Technologies, Inc.), with the pRSC-LacZ constructs in the presence or absence of different apoptosis-inhibitors. 30 h after transfection cells were stained with β -galactosidase and examined for morphological signs of apoptosis. The percentage of round blue apoptotic cells (mean \pm SD) were represented as a function of total blue
25 cells under each condition ($n \geq 3$).

EXAMPLE 3

IN VITRO TRANSLATION OF CASPASES

³⁵S-labeled caspases (wild-type and rev-caspases) were obtained by *in vitro* translation in the presence of ³⁵S-methionine using a coupled transcription/translation system in rabbit reticulocyte lysate using TNT Kit (Promega) according to the manufacturer's recommendations. Unlike the wild-type caspase-3 and 6, Figure 2A demonstrates that rev-caspase-3 and 6 were able to undergo autocatalytic processing in the *in vitro* translation reaction. Further, this processing was completely inhibited by mutation of the active site Cys of rev-caspase-3 and 6 (Figure 2A, lanes 3 and 6) and by selected caspase inhibitors (See Example 4). Because the *in vitro* translated products are present at very low concentration in the reaction mixture, the observed cleavage must be attributed to an intramolecular processing within the caspase heterotetramer.

15

EXAMPLE 4

EFFECTS OF INHIBITORS ON REV-CASPASE-3 AND 6 ACTIVITY

To test the effect of selected caspase inhibitors on the autocatalytic activity of rev-caspase-3 and 6, the rev-caspases were translated as in Example 3, but in the presence of varying amounts of inhibitors. As demonstrated by Figure 2B, in the presence of increasing amounts of DEVD-CHO (SEQ ID NO:52), a decrease in the amount of cleavage products and a corresponding increase in the amount of the revcaspase-3 precursor was observed. This corresponded to nearly 50-90% inhibition of the autocatalytic activity of rev-caspase-3 at 40-400 nM concentration. However, the same concentrations of this inhibitor had little effect on the autocatalytic activity of rev-caspase-6 (Figure 2C). This is consistent with earlier observations that caspase-6 is poorly inhibited by DEVD-CHO (SEQ ID NO:52); see Srinivasula et al., *J. Biol. Chem.*, 271:27099-27106, 1996. On the other hand, as is apparent from

30

inspection of Figures 2B and 2C, z-VAD-fmk had nearly an equal inhibitory effect on rev-caspase-3 and 6 autocatalytic activity at the concentration used in this experiment. Nevertheless, nearly 10-fold more of z-VAD-fmk than DEVD-CHO (SEQ ID NO:52) was required to obtain complete inhibition of caspase-3 activity. Similarly, baculovirus p35 had nearly an equal inhibitory effect on rev-caspase-3 and 6 autocatalytic activity (data not shown).

EXAMPLE 5

10 CASPASE-3 AND 6 SPECIFICITY RETENTION OF BACTERIALLY EXPRESSED REV-CASPASE-3 AND -6

At limited caspase concentrations, poly(ADP) ribose polymerase (PARP) is specifically cleaved by caspase-3 and 7 but not other caspases. Similarly, 15 lamin is specifically cleaved by caspase-6 but not other caspases. To compare the activity of the wild-type and rev-caspase-3 and 6 towards PARP and lamin, rev-caspase-3 and 6 were expressed in bacteria and then incubated with the two substrates PARP and lamin. As shown in Figures 3A and B, the activity of the rev-caspases towards these two substrates were indistinguishable from their wild-type 20 counterparts. Both caspase-3 variants (rev and WT), but not caspase-6 variants efficiently cleaved PARP. In contrast, both caspase-6 variants, but not caspase-3 variants efficiently cleaved lamin. These results demonstrate that the mature caspases generated from the rev and the wild type constructs have identical substrate specificity.

EXAMPLE 6

INDUCTION OF APOPTOSIS IN MAMMALIAN CELLS BY REV-CASPASE-3 AND -6

To determine the apoptotic activity of rev-caspase-3 and 6 *in vivo*, the
5 rev-caspases were expressed in human MCF-7 cells, transfected as explained above in
Example 2. As evidenced by Figures 4A and B, unlike the wild type caspase-3 and 6,
the rev-caspases potently induced apoptosis in nearly 90% of the transfected cells.
Overexpression of Bcl-2 or CrmA, which protect against different forms of
apoptosis, did not significantly reduce their apoptotic activity. Nevertheless,
10 overexpression of the baculovirus p35, which inhibits the activity of most caspases,
partially protected against their apoptotic activity. Also, incubation of the transfected
cells in the presence of 100 μ M z-VAD-fmk, dramatically reduced their apoptotic
activity to nearly 30%. These data demonstrate directly that the activity of caspase-3
and 6 are downstream of the CrmA and Bcl-2 block in the apoptotic cascade, and can
15 only be inhibited by high concentration of the pancaspase-inhibitor z-VAD-fmk.

EXAMPLE 7

ACTIVITY OF NONCLEAVABLE REV-CASPASE-3

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To demonstrate that the rev-caspase molecules are inherently active
and do not require separation of the two subunits and that the two subunits are
derived from the same contiguous molecule, the DEVD (SEQ ID NO:52) site was
removed and Asp9 and 28, that are present between the two subunits of
25 rev-caspase-3, were mutated (see Figure 2A). However, to follow the activity of this
molecule a cleavable 35 residue long His6-T7-tag N-terminal to the IETD (SEQ ID
NO:53) site was introduced (see Figure 1B). Figure 5 demonstrates that upon *in vitro*
translation of this molecule, as described in Example 3 above, there was no evidence
of cleavage between the two subunits. Nevertheless, the translated molecule was
30 active as evident from its ability to cleave its T7-tag to form the p32 species (Figure

5). In the presence of 400 nM DEVD-CHO (SEQ ID NO:52), processing of the T7-tag was inhibited and only the full length p34 species can be seen. Furthermore, expression of this molecule into MCF-7 cells potently induced apoptosis in these cells. These data demonstrate that when the two subunits of a caspase are rearranged in the reverse order, it is not necessary to separate them from each other to generate an active caspase. Thus by mimicking the mature caspase structure, it is possible to design a contiguous active caspase molecules.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

WE CLAIM:

1. An isolated nucleic acid molecule encoding a rev-caspase.
2. The nucleic acid molecule of claim 1, wherein the rev-caspase is selected from the group consisting of rev-caspase-1, rev-caspase-2, rev-caspase-3, rev-caspase-4, rev-caspase-5, rev-caspase-6, rev-caspase-7, rev-caspase-8, rev-caspase-9, rev-caspase-10, rev-caspase-11, rev-caspase-12, rev-caspase-13, and rev-caspase-14.
3. The nucleic acid molecule of claim 1, wherein the rev-caspase is a human rev-caspase.
4. The nucleic acid molecule of claim 1, wherein the rev-caspase is human rev-caspase-3.
5. The nucleic acid molecule of claim 4, wherein human rev-caspase-3 comprises the sequence recited in Figure 21A (SEQ ID NO:34) or Figure 21B (SEQ ID NO:35) or variants thereof.
6. The nucleic acid molecule of claim 4, wherein human rev-caspase-3 is encoded by the sequence in Figure 7 (SEQ ID NO:1) or Figure 8 (SEQ ID NO:2) or variants thereof.
7. The nucleic acid molecule of claim 1, wherein the rev-caspase is human rev-caspase-6.
8. The nucleic acid molecule of claim 7, wherein human rev-caspase-6 comprises the sequence in Figure 21C (SEQ ID NO:36) or variants thereof.

9. The nucleic acid molecule of claim 7, wherein human rev-caspase-6 is encoded by the sequence in Figure 9 (SEQ ID NO: 3) or variants thereof.
10. An expression vector comprising the nucleic acid molecule of any one of claims 1-9, wherein the sequence encoding rev-caspase is operatively linked to a promoter.
11. The expression vector of claim 10, wherein the promoter is inducible.
12. The expression vector of claim 11, wherein the inducible promoter is HIV LTR.
13. A host cell transfected with the expression vector of claim 10.
14. The host cell of claim 13, wherein the cell is a bacterium or a mammalian cell.
15. A rev-caspase protein.
16. The rev-caspase protein of claim 15, wherein the rev-caspase is selected from the group consisting of rev-caspase-1, rev-caspase-2, rev-caspase-3, rev-caspase-4, rev-caspase-5, rev-caspase-6, rev-caspase-7, rev-caspase-8, rev-caspase-9, rev-caspase-10, rev-caspase-11, rev-caspase-12, rev-caspase-13, and rev-caspase-14.
17. The rev-caspase protein of claim 15, wherein the rev-caspase is a human rev-caspase.
18. The rev-caspase protein of claim 15, wherein the rev-caspase is human rev-caspase-3.

19. The rev-caspase protein of claim 18, wherein human rev-caspase-3 comprises the sequence in Figure 21A (SEQ ID NO:34) or Figure 21B (SEQ ID NO:35) or variants thereof.

20. The rev-caspase protein of claim 18, wherein human rev-caspase-3 is encoded by the sequence in Figure 7 (SEQ ID NO:1) or Figure 8 (SEQ ID NO:2) or variants thereof.

21. The rev-caspase protein of claim 15, wherein the rev-caspase is human rev-caspase-6.

22. The rev-caspase protein of claim 21, wherein human rev-caspase-6 comprises the sequence in Figure 21C (SEQ ID NO:36) or variants thereof.

23. The rev-caspase protein of claim 21, wherein human rev-caspase-6 is encoded by the sequence in Figure 9 (SEQ ID NO:3) or variants thereof.

24. A method of identifying an inhibitor or enhancer of caspase processing activity, comprising:

(a) contacting a sample containing an *in vitro* translated rev-caspase with a candidate inhibitor or candidate enhancer; and

(b) detecting the presence of large and small subunits of rev-caspase, and therefrom determining the level of caspase processing activity, wherein a decrease in processing indicates the presence of a caspase inhibitor, and wherein an increase in processing indicates the presence of a caspase enhancer, wherein processed rev-caspase yields large and small subunits.

25. A method of identifying an inhibitor or enhancer of caspase processing activity, comprising:

(a) contacting a cell transfected with the vector expressing rev-caspase according to claim 10 with a candidate inhibitor or candidate enhancer; and

(b) detecting the presence of large and small subunits of rev-caspase, and therefrom determining the level of caspase processing activity, wherein a decrease in processing indicates the presence of a caspase inhibitor, and wherein an increase in processing indicates the presence of a caspase enhancer, wherein processed rev-caspase yields large and small subunits.

26. A method of identifying an inhibitor or enhancer of caspase-mediated apoptosis, comprising:

(a) contacting a cell transfected with the vector expressing rev-caspase according to claim 10 with a candidate inhibitor or candidate enhancer or with a reference compound; and

(b) detecting cell viability,

wherein viability of cells contacted with a candidate is increased in the presence of an inhibitor and is decreased in the presence of an enhancer compared to cells contacted with a reference compound.

27. The method of any one of claims 24-26, wherein the rev-caspase is selected from the group consisting of rev-caspase-1, rev-caspase-2, rev-caspase-3, rev-caspase-4, rev-caspase-5, rev-caspase-6, rev-caspase-7, rev-caspase-8, rev-caspase-9, rev-caspase-10, rev-caspase-11, rev-caspase-12, rev-caspase-13, and rev-caspase-14.

28. The method of either of claims 24 or 25, wherein detecting comprises gel electrophoresis.

29. The method of claim 24, wherein the *in vitro* translated rev-caspase is labeled.

30. The method of claim 29, wherein the label is a radioactive label, a peptide tag, an enzyme or biotin.

31. A gene delivery vehicle, comprising the nucleic acid molecule according to any one of claims 1-9, wherein the rev-caspase sequence is operatively linked to a promoter.

32. The gene delivery vehicle of claim 31, wherein the vehicle is a retrovirus or adenovirus.

33. The gene delivery vehicle of claim 31, wherein the nucleic acid molecule is associated with a polycation.

34. The gene delivery vehicle of any one of claims 31-33, further comprising a ligand that binds a cell surface receptor.

35. A method of treating cancer, comprising administering to a patient the gene delivery vehicle according to any one of claims 31-34, wherein the gene delivery vehicle is internalized by tumor cells.

36. A method of treating autoimmune disease, comprising administering to a patient the gene delivery vehicle according to any one of claims 31-34, wherein the gene delivery vehicle is internalized by cells mediating autoimmune disease.

Fig. 1A

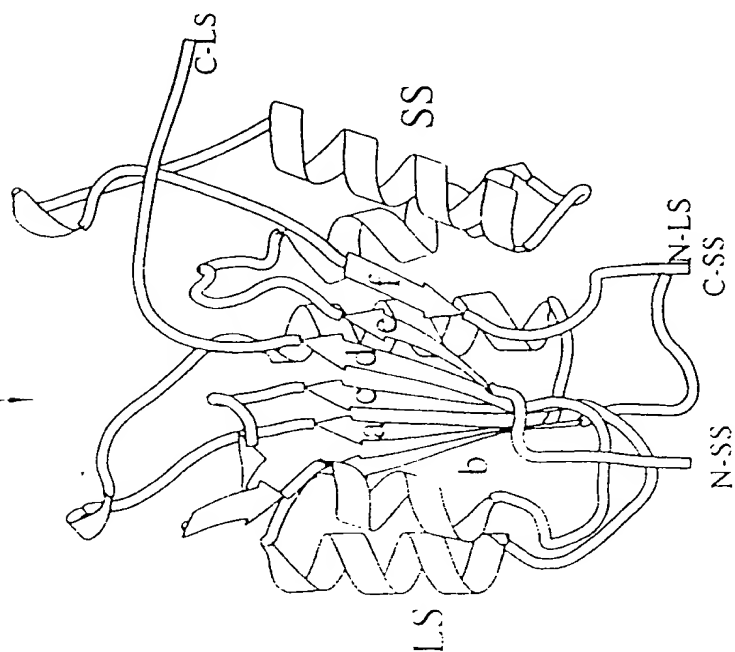
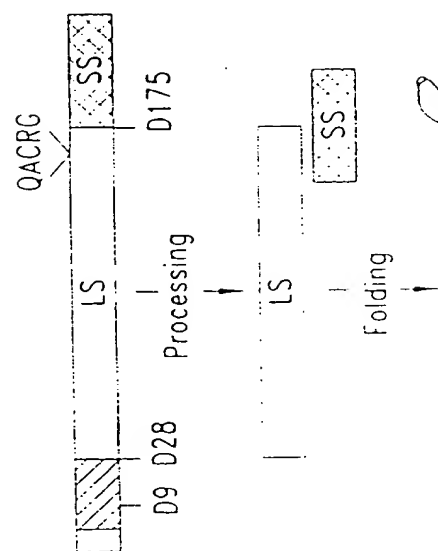


Fig. 1B

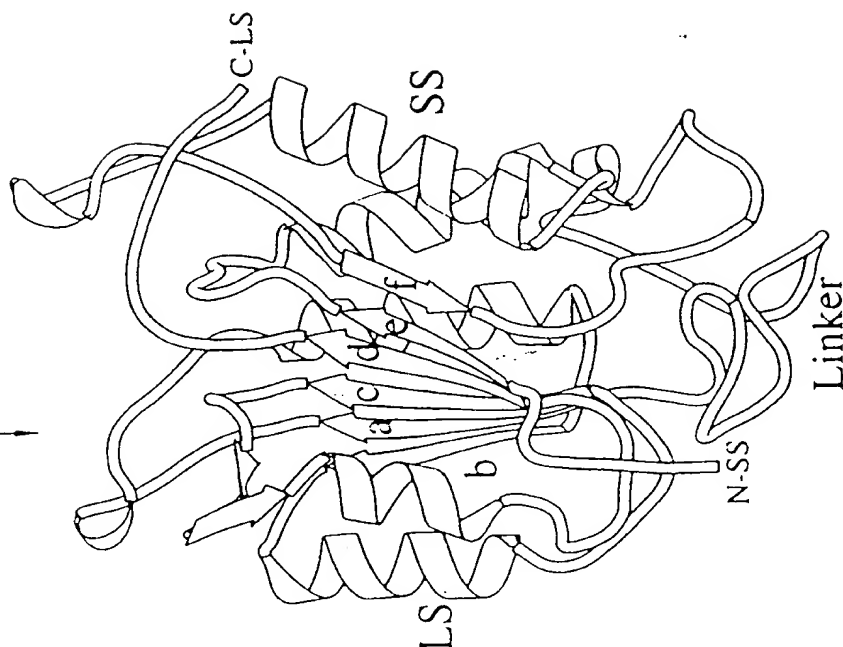
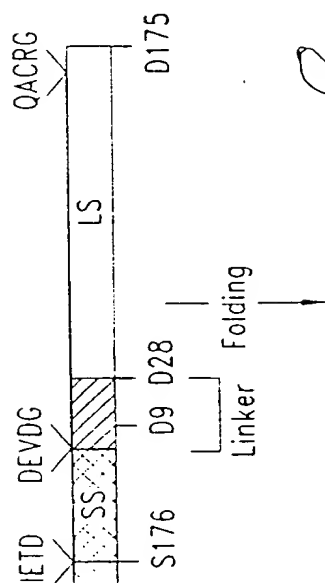
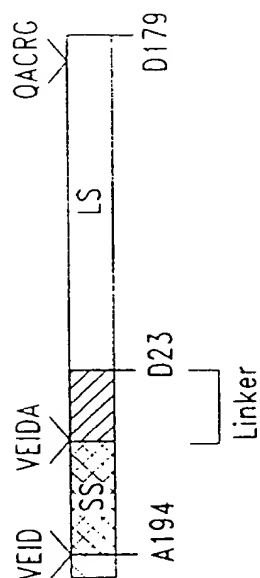


Fig. 1C



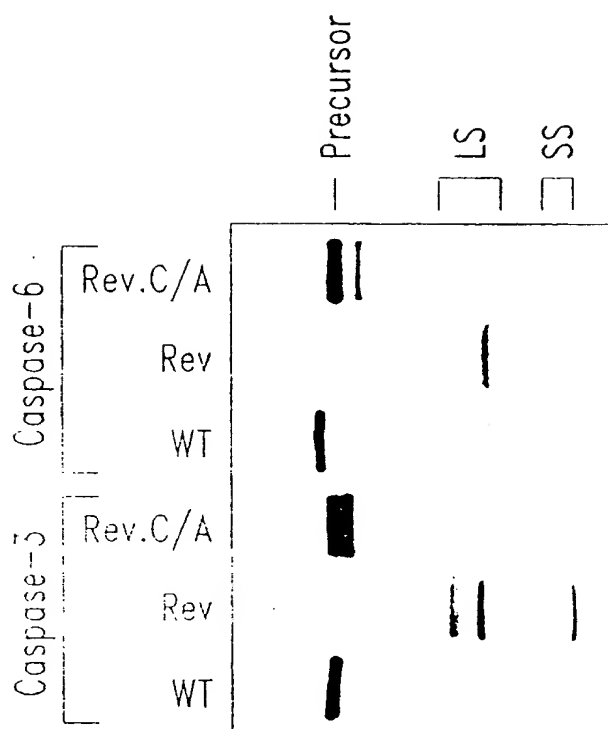
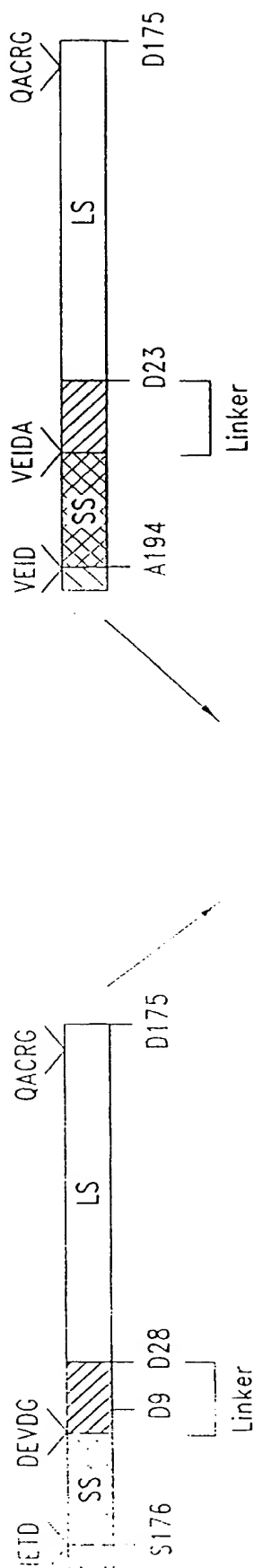


Fig. 2A

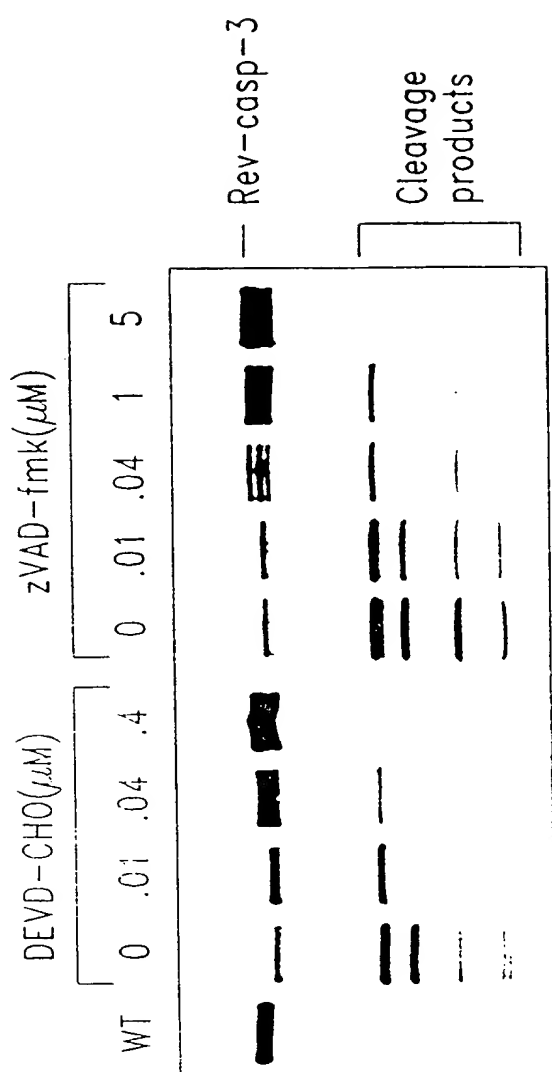


Fig. 2B

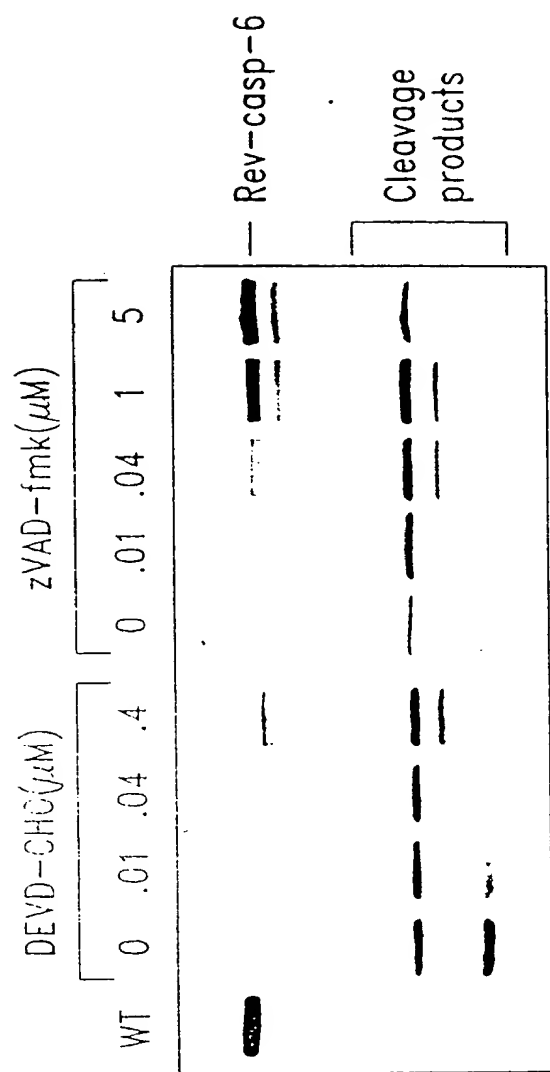
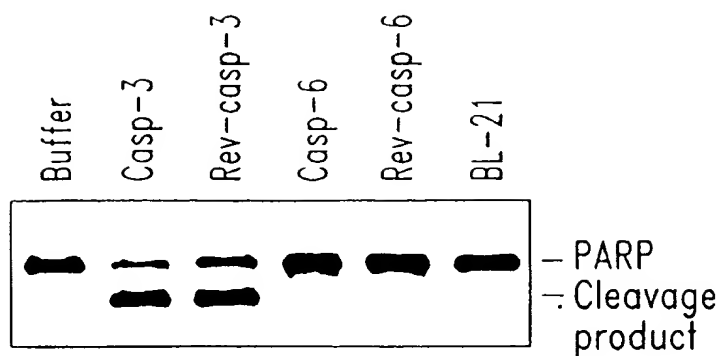
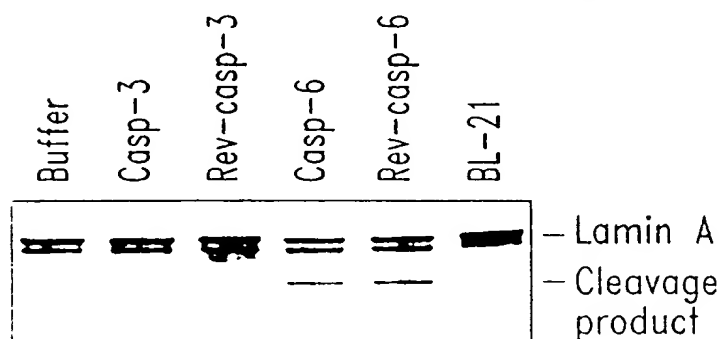
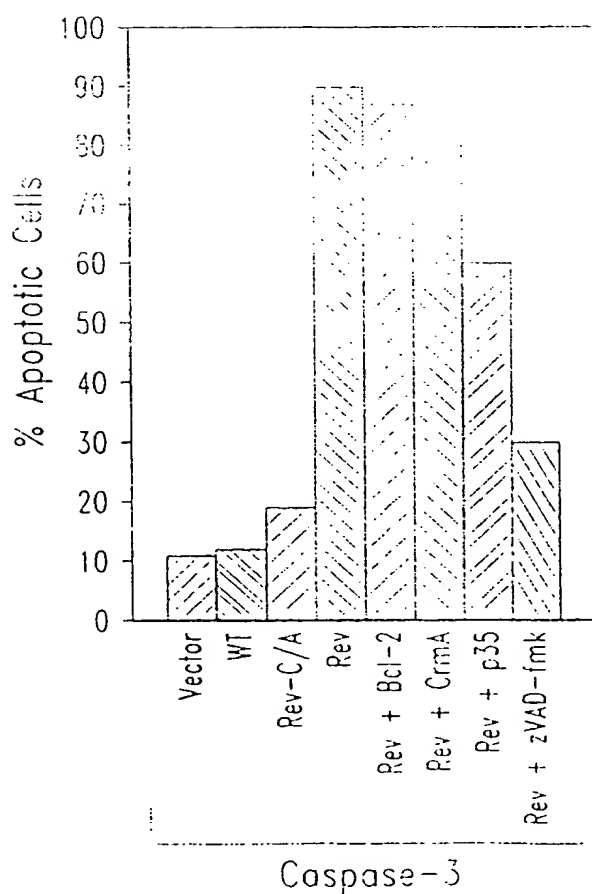
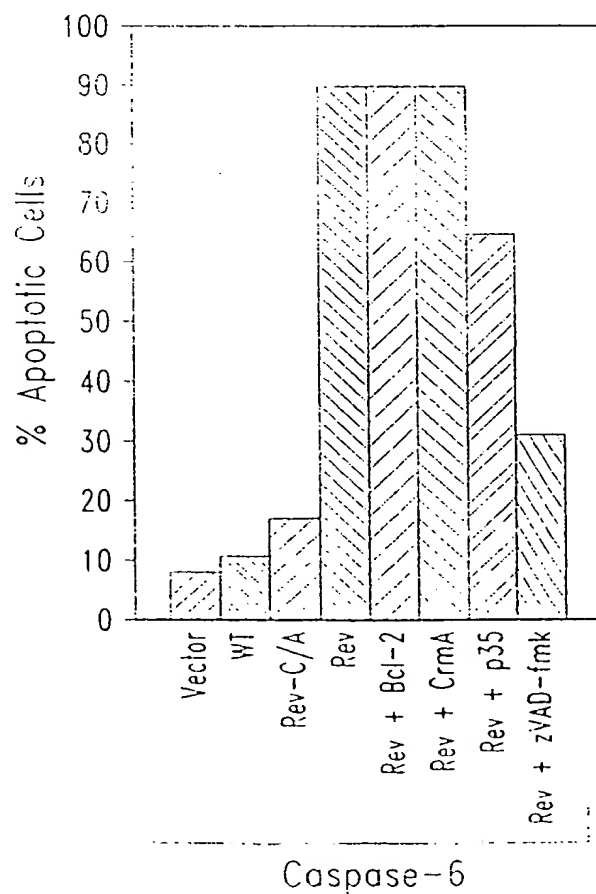


Fig. 2C

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Fig. 3A*Fig. 3B**Fig. 4A**Fig. 4B*

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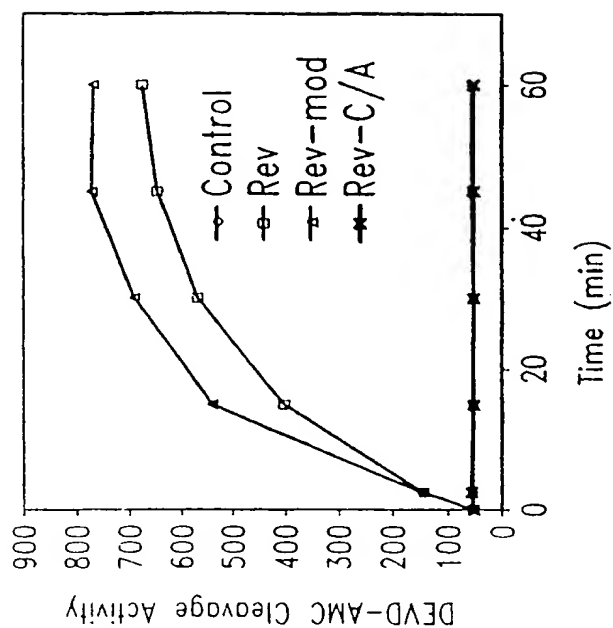
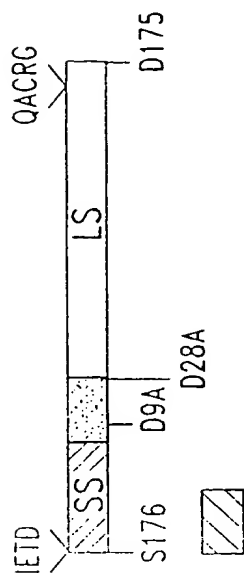


Fig. 5B

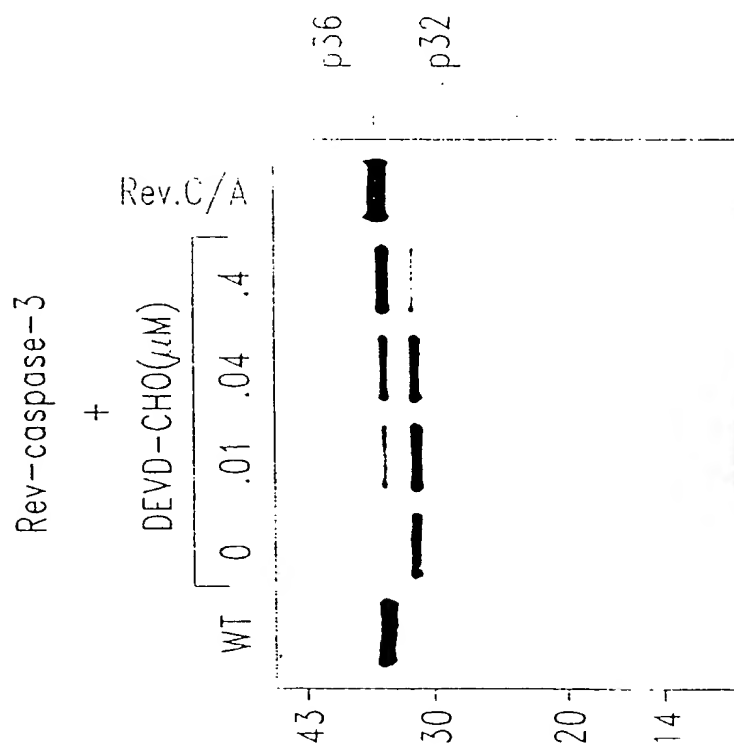
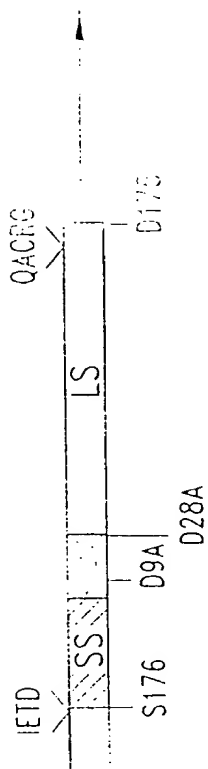


Fig. 5A

Large Subunit		Small Subunit	
*			
Mch6	178 RTRTGS..LSHGCG..FIQACGGEQ..PEPDA..DQLDA..GFVSWRDPKSGSWYV		
Mch5	275 RDRNGT..LSHGDK..FIQACQGDN..VETDS..LEMDL..NCVSYRNPAGTWYI		
Mch4	255 KDRQGT..LTHGRF..FIQACGEE..IEADA.....GYVSFRHVEEGSWYI		
Mch3	85 GVRNGT..LSHGEE..FIQACRGTE..IQADS.....GYYSWRSPGRGSWFV		
Mch2	62 PERRGT..LSHGEG..IIQACRGNQ..DVVDN..TEVDA..GYSHRETIVNGSWYI		
CPP32	62 TSRSQT..LSHGEE..IIQACRGTE..IETDS.....GYYSWRNSKDGSWFI		
CED-3	257 PTRNGT..LSHGEE..FVQACRGER..DSVDG.....QYVSWRNSARGSWFI		
ICL	177 PRRTGA..MSGIR..IIQACRGDS..WFKDS..FEDDA..DNVSWRHPTMGSVFI		
IX	150 PPRNGA..MSGIL..IVQACRGAN..WVKDS..LEEDA..HNVSWRDSITMGSIPI		
ICeRe1111	191 PARNGA..MSGIL..IVQACRGEK..WVRDS..LEADS..HNVSWRDRTRGSIPI		
ICH 1	200 EFRSGG..LSHGVE..FIQACRGDE..DQDDG..EESDA..GTAAMRNTKRGSWYI		
	b acc bac DX DX		aaabaa a ba

Fig. 6

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Fig. 7

Rev-Caspase-3
Translation start 1
Translation stop 873

1 ATGATTGAGA CAGACAGTGG TGTTGATGAT GACATGGCGT GTCATAAAAT
51 ACCAGTGGAG GCCGACTTCT TGTATGCATA CTCCACAGCA CCTGGTTATT
101 ATTCTTGGCG AAATTCAAAG GATGGCTCCT GGTTTCATCCA GTCGCTTTGT
151 GCCATGCTGA AACAGTATGC CGACAAGCTT GAATTTATGC ACATTCTTAC
201 CCGGGTTAAC CGAAAGGTGG CAACAGAATT TGAGTCCTTT TCCTTTGACG
251 CTACTTTTCA TGCAAAGAAA CAGATTCCAT GTATTGTTTC CATGCTCACA
301 AAAGAACTCT ATTTTTATCA CGATGAAGTT GATGGGGGAT CCCCCATGGA
351 GAACACTGAA AACTCAGTGG ATTCAAAATC CATTAAAAAT TTGGAACCAA
401 AGATCATACA TGAAGCGAA TCAATGGACT CTGGAATATC CCTGGACAAC
451 AGTTATAAAA TGGATTATCC TGAGATGGGT TTATGTATAA TAATTAATAA
501 TAAGAATTTT CATAAGAGCA CTGGAATGAC ATCTCGGTCT GGTACAGATG
551 TCGATGCAGC AAACCTCAGG GAAACATTCA GAAACTTGAA ATATGAAGTC
601 AGGAATAAAA ATGATCTTAC ACGTGAAGAA ATTGTGGAAT TGATGCGTGA
651 TGTTTCTAAA GAAGATCACA GCAAAAGGAG CAGTTTTGTT TGTGTGCTTC
701 TGAGCCATGG TGAAGAAGGA ATAATTTTTG GAACAAATGG ACCTGTTGAC
751 CTGAAAAAAA TAACAACTT TTTTCAGAGGG GATCGTTGTA GAAGTCTAAC
801 TGGAAAACCC AAACTTTTCA TTATTCAGGC CTGCCGTGGT ACAGAACTGG
851 ACTGTGGCAT TGAGACAGAC TGA

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Fig. 8

Uncleavable Rev-caspase-3
Translation start 1
Translation stop 858

1 ATGATTGAGA CAGACAGTGG TGTTGATGAT GACATGGCGT GTCATAAAAT
51 ACCAGTGGAG GCCGACTTCT TGTATGCATA CTCCACAGCA CCTGGTTATT
101 ATTCTTGGCG AAATTCAAAG GATGGCTCCT GGTTCATCCA GTCGCTTTGT
151 GCCATGCTGA AACAGTATGC CGACAAGCTT GAATTTATGC ACATTCTTAC
201 CCGGGTTAAC CGAAAGGTGG CAACAGAATT TGAGTCCTTT TCCTTTGACG
251 CTACTTTTCA TGCAAAGAAA CAGATTCCAT GTATTGTTTC CATGCTCACA
301 AAAGAACTCT ATTTTTATCA CGGATCCCCC ATGGAGAACA CTGAAAACCTC
351 AGTGGCTTCA AAATCCATTA AAAATTTGGA ACCAAAGATC ATACATGGAA
401 GCGAATCAAT GGCCTCTGGA ATATCCCTGG ACAACAGTTA TAAAATGGAT
451 TATCCTGAGA TGGGTTTATG TATAATAATT AATAATAAGA ATTTTCATAA
501 GAGCACTGGA ATGACATCTC GGTCTGGTAC AGATGTGAT GCAGCAAACC
551 TCAGGGAAAC ATTCAGAAAC TTGAAATATG AAGTCAGGAA TAAAAATGAT
601 CTTACACGTG AAGAAATTGT GGAATTGATG CGTGATGTTT CTAAAGAAGA
651 TCACAGCAAA AGGAGCAGTT TTGTTTGTGT GCTTCTGAGC CATGGTGAAG
701 AAGGAATAAT TTTTGGAAACA AATGGACCTG TTGACCTGAA AAAAATAACA
751 AACTTTTTCA GAGGGGATCG TTGTAGAAGT CTAAGTGGAA AACCCAAACT
801 TTTCATTATT CAGGCCTGCC GTGGTACAGA ACTGGACTGT GGCATTGAGA
851 CAGACTGA

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Fig. 9

Rev-caspase-6
Translation start 1
Translation stop 903

1 ATGGTAGAAA TAGATGCAGC CTCCGTTTAC ACGCTGCCTG CTGGAGCTGA
51 CTTCTCATG TGTTACTCTG TTGCAGAAGG ATATTATTCT CACCGGGAAA
101 CTGTGAACGG CTCATGGTAC ATTCAAGATT TGTGTGAGAT GTTGGGAAAA
151 TATGGCTCCT CCTTAGAGTT CACAGAACTC CTCACACTGG TGAACAGGAA
201 AGTTTCTCAG CGCCGAGTGG ACTTTTGCAA AGACCCAAGT GCAATTGGAA
251 AGAAGCAGGT TCCCTGTTTT GCCTCAATGC TAACTAAAAA GCTGCATTTC
301 TTTCCAAAAT CTAATCTCGA GCACCACCAC CACCACCACG TTGAAATTGA
351 TGGGGGATCC CCCATGAGCT CGGCCTCGGG GCTCCGCAGG GGGCACCCGG
401 CAGGTGGGGA AGAAAACATG ACAGAAACAG ATGCCTTCTA TAAAAGAGAA
451 ATGTTTGATC CGGCAGAAAA GTACAAAATG GACCACAGGA GGAGAGGAAT
501 TGCTTTAATC TTCAATCATG AGAGGTTCTT TTGGCACTTA AACTGCCAG
551 AAAGGCGGGG CACCTGCGCA GATAGAGACA ATCTTACCCG CAGGTTTTCA
601 GATCTAGGAT TTGAAGTGAA ATGCTTTAAT GATCTTAAAG CAGAAGAACT
651 ACTGCTCAAA ATTCATGAGG TGTCAACTGT TAGCCACGCA GATGCCGATT
701 GCTTTGTGTG TGTCTTCCTG AGCCATGGCG AAGGCAATCA CATTTATGCA
751 TATGATGCTA AAATCGAAAT TCAGACATTA ACTGGCTTGT TCAAAGGAGA
801 CAAGTGTCAC AGCCTGGTTG GAAAACCCAA GATATTTATC ATCCAGGCAT
851 GTCGGGGAAA CCAGCACGAT GTGCCAGTCA TTCCTTTGSA TGTAGTAGAT
901 TAA

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I	SS	P	LS
SS	P	LS	
SS	X	LS	

Fig. 10

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Fig. 11A

CASPASE 1

```

+1 MetAlaAspLysValLeuLysGluLysArgLysLeuPheIleArgSerMetGlyGluGly
1 ATGGCCGACAAGGTCCTGAAGGAGAAGAGAAAAGCTGTTTATCCGTTCCATGGGTGAAGGT
TACCGGCTGTTCCAGGACTTCCTCTTCTCTTTGACAAATAGGCAAGGTACCCACTTCCA
+1 ThrIleAsnGlyLeuLeuAspGluLeuLeuGlnThrArgValLeuAsnLysGluGluMet
61 ACAATAAATGGCTTACTGGATGAATTATTACAGACAAGGGTGCTGAACAAGGAAGAGATG
TGTTATTTACCGAATGACCTACTTAATAATGTCTGTTCCACGACTTGTTCTTCTCTAC
+1 GluLysValLysArgGluAsnAlaThrValMetAspLysThrArgAlaLeuIleAspSer
121 GAGAAAGTAAAACGTGAAAATGCTACAGTTATGGATAAGACCCGAGCTTTGATTGACTCC
CTCTTTTCATTTTGCACCTTTACGATGTCAATACCTATTCTGGGCTCGAAACTAACTGAGG
+1 ValIleProLysGlyAlaGlnAlaCysGlnIleCysIleThrTyrIleCysGluGluAsp
181 GTTATTCCGAAAGGGGCACAGGCATGCCAAATTTGCATCACATACATTTGTGAAGAAGAC
CAATAAGGCTTTCCCGTGTCCGTACGGTTTAAACGTAGTGTATGTAAACACTTCTTCTG
+1 SerTyrLeuAlaGlyThrLeuGlyLeuSerAlaAspGlnThrSerGlyAsnTyrLeuAsn
241 AGTTACCTGGCAGGGACGCTGGGACTCTCAGCAGATCAAACATCTGGAAATTACCTTAAT
TCAATGGACCGTCCCTGCGACCTGAGAGTCGTCTAGTTTGTAGACCTTTAATGGAATTA
+1 MetGlnAspSerGlnGlyValLeuSerSerPheProAlaProGlnAlaValGlnAspAsn
301 ATGCAAGACTCTCAAGGAGTACTTTCTTCCTTTCCAGCTCCTCAGGCAGTGCAGGACAAC
TACGTTCTGAGAGTTCTCATGAAAGAAGGAAAGGTGCGAGGAGTCCGTCACGTCCTGTTG
+1 ProAlaMetProThrSerSerGlySerGluGlyAsnValLysLeuCysSerLeuGluGlu
361 CCAGCTATGCCACATCCTCAGGCTCAGAAGGGAATGTCAAGCTTTGCTCCCTAGAAGAA
GGTCGATACGGGTGTAGGAGTCCGAGTCTTCCCTTACAGTTCGAAACGAGGGATCTTCTT
+1 AlaGlnArgIleTrpLysGlnLysSerAlaGluIleTyrProIleMetAspLysSerSer
421 GCTCAAAGGATATGGAAACAAAAGTCGGCAGAGATTTATCCAATAATGGACAAGTCAAGC
CGAGTTTCTTATACCTTTGTTTTAGCCGTCCTCTAAATAGGTTATTACCTGTTCAAGTTCG
+1 ArgThrArgLeuAlaLeuIleIleCysAsnGluGluPheAspSerIleProArgArgThr
481 CGCACACGTCTTGCTCTCATTATCTGCAATGAAGAATTTGACAGTATTCCTAGAAGAACT
CGGTGTGCAGAACGAGAGTAATAGACGTTACTTCTTAAACTGTGATAAGGATCTTCTTGA
+1 GlyAlaGluValAspIleThrGlyMetThrMetLeuLeuGlnAsnLeuGlyTyrSerVal
541 GGAGCTGAGGTTGACATCACAGGCATGACAATGCTGCTACAAAATCTGGGGTACAGCGTA
CCTCGACTCCAACTGTAGTGTCCGTACTGTTAGGAGGATGTTTAGACCCCATGTGGCAT
+1 AspValLysLysAsnLeuThrAlaSerAspMetPheThrGluLeuGluAlaPheAlaIle
601 GATGTGAAAAA--AATCTCACTGCTTCGGACATGCTT--GAGAGCTGGAGGGCAATTTGCACAC
CTACACTTTTTTTT--AGGTGACGAAGCCTGTACTTATTTCTCGACCTCCGTAAACCTGTG

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Fig. 11B

CASPASE 1

```

+1 ArgProGluHisLysThrSerAspSerThrPheLeuValPheMetSerHisGlyIleArg
-----
661 CGCCAGAGCACAAGACCTCTGACAGCACGTTCTGGTGTTCATGTCTCATGGTATTCGG
GCGGGTCTCGTGTTCTGGAGACTGTCGTGCAAGGACCACAAGTACAGAGTACCATAAGCC
-----
+1 GluGlyIleCysGlyLysLysHisSerGluGlnValProAspIleLeuGlnLeuAsnAla
-----
721 GAAGGCATTTGTGGGAAGAAACACTCTGAGCAAGTCCCAGATATACTACAACCTCAATGCA
CTTCCGTAAACACCCTTCTTTGTGAGACTCGTTCAGGGTCTATATGATGTTGAGTTACGT
-----
+1 IlePheAsnMetLeuAsnThrLysAsnCysProSerLeuLysAspLysProLysValIle
-----
781 ATCTTTAACATGTTGAATACCAAGAACTGCCCAAGTTTGAAGGACAAACCGAAGGTGATC
TAGAAATTGTACAACCTATGGTTCTTGACGGGTCAAACCTCCTGTTTGGCTTCCACTAG
-----
+1 IleIleGlnAlaCysArgGlyAspSerProGlyValValTrpPheLysAspSerValGly
-----
841 ATCATCCAGGCCTGCCGTGGTGACAGCCCTGGTGTGGTGTGGTTTAAAGATTTCAGTAGGA
TAGTAGGTCCGGACGGCACCCTGTCTGGGACCACACCACACCAAATTTCTAAGTCATCCT
-----
+1 ValSerGlyAsnLeuSerLeuProThrThrGluGluPheGluAspAspAlaIleLysLys
-----
901 GTTTCTGGAAACCTATCTTTACCAACTACAGAAGAGTTTGAGGATGATGCTATTAAGAAA
CAAAGACCTTTGGATAGAAATGGTTGATGTCTTCTCAAACCTCCTACTACGATAATTCTTT
-----
+1 AlaHisIleGluLysAspPheIleAlaPheCysSerSerThrProAspAsnValSerTrp
-----
961 GCCCACATAGAGAAGGATTTTATCGCTTTCTGCTCTTCCACACCAGATAATGTTTCTTGG
CGGGTGTATCTCTTCTTAAATAGCGAAAGACGAGAAGGTGTGGTCTATTACAAAGAACC
-----
+1 ArgHisProThrMetGlySerValPheIleGlyArgLeuIleGluHisMetGlnGluTyr
-----
1021 AGACATCCCACAATGGGCTCTGTTTTTATTGGAAGACTCATTGAACATATGCAAGAATAT
TCTGTAGGGTGTACCCGAGACAAAAATAACCTTCTGAGTAACTTGTATACGTTCTTATA
-----
+1 AlaCysSerCysAspValGluGluIlePheArgLysValArgPheSerPheGluGlnPro
-----
1081 GCCTGTTCTGTGATGTGGAGGAAATTTCCGCAAGGTTTCGATTTTCATTTGAGCAGCCA
CGGACAAGGACACTACACCTCCTTTAAAGGCGTTCCAAGCTAAAAGTAACTCGTCGGT
-----
+1 AspGlyArgAlaGlnMetProThrThrGluArgValThrLeuThrArgCysPheTyrLeu
-----
1141 GATGGTAGAGCGCAGATGCCACCACTGAAAGAGTGACTTTGACAAGATGTTTCTACCTC
CTACCATCTCGCGTCTACGGGTGGTACTTTCTCACTGAAACTGTTCTACAAAGATGGAG
-----
+1 PheProGlyHis
-----
>
1201 TTCCCAGGACATTAA
AAGGGTCCTGTAATT

```

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Fig. 12A

CASPASE 2

+1	MetAlaAlaAspArgGlyArgArgIleLeuGlyValCysGlyMetHisProHisHisGln
1	ATGGCCGCTGACAGGGGACGCAGGATATTGGGAGTGTGTGGCATGCATCCTCATCATCAG TACCGGCGACTGTCCCCTGCGTCTATAACCCCTCACACACCGTACGTAGGAGTAGTAGTC
+1	GluThrLeuLysLysAsnArgValValLeuAlaLysGlnLeuLeuLeuSerGluLeuLeu
61	GAAACTCTAAAAAAGAACCGAGTGGTGTAGCCAAACAGCTGTTGTTGAGCGAATTGTTA CTTTGAGATTTTTTCTTGGCTCACCACGATCGGTTTGTGCGACAACAACTCGCTTAACAAT
+1	GluHisLeuLeuGluLysAspIleIleThrLeuGluMetArgGluLeuIleGlnAlaLys
121	GAACATCTTCTGGAGAAGGACATCATCACCTTGGAAATGAGGGAGCTCATCCAGGCCAAA CTTGTAGAAGACCTCTTCCTGTAGTAGTGGAACCTTTACTCCCTCGAGTAGGTCCGGTTT
+1	ValGlySerPheSerGlnAsnValGluLeuLeuAsnLeuLeuProLysArgGlyProGln
181	GTGGGCAGTTTCAGCCAGAATGTGGAACCTCAACTTGCTGCCTAAGAGGGGTCCCCAA CACCCGTCAAAGTCGGTCTTACACCTTGAGGAGTTGAACGACGGATTCTCCCCAGGGGTT
+1	AlaPheAspAlaPheCysGluAlaLeuArgGluThrLysGlnGlyHisLeuGluAspMet
241	GCTTTTGTATGCCTTCTGTGAAGCACTGAGGGAGACCAAGCAAGGCCACCTGGAGGATATG CGAAACTACGGAAGACACTTCGTGACTCCCTCTGGTTCGTTCCGGTGGACCTCCTATAC
+1	LeuLeuThrThrLeuSerGlyLeuGlnHisValLeuProProLeuSerCysAspTyrAsp
301	TTGCTCACCACCCTTTCTGGGCTTCAGCATGTACTCCCACCGTTGAGCTGTGACTACGAC AACGAGTGGTGGGAAAGACCCGAAGTCGTACATGAGGGTGGCAACTCGACACTGATGCTG
+1	LeuSerLeuProPheProValCysGluSerCysProLeuTyrLysLysLeuArgLeuSer
361	TTGAGTCTCCCTTTTCCGGTGTGTGAGTCCTGTCCCTTTACAAGAAGCTCCGCCTGTG AACTCAGAGGGGAAAAGGCCACACACTCAGGACAGGGGAAATGTTCTTCGAGGCGGACAGC
+1	ThrAspThrValGluHisSerLeuAspAsnLysAspGlyProValCysLeuGlnValLys
421	ACAGATACTGTGGAACACTCCCTAGACAATAAAGATGGTCCTGTCTGCCTTCAGGTGAAG TGTCTATGACACCTTGTGAGGGATCTGTTATTTCTACCAGGACAGACGGAAGTCCACTTC
+1	ProCysThrProGluPheTyrGlnThrHisPheGlnLeuAlaTyrArgLeuGlnSerArg
481	CCTTGCACTCCTGAATTTTATCAAACACACTTCCAGCTGGCATATAGGTTGCAGTCTCGG GGAACGTGAGGACTTAAAATAGTTTGTGTGAAGGTGACCGTATATCCAACGTGAGAGCC
+1	ProArgGlyLeuAlaLeuValLeuSerAsnValHisPheThrGlyGluLysGluLeuGlu
541	CCTCGTGGCCTAGCACTGGTGTGAGCAATGTGCACTTCACTGGAGAGAAAGAACTGGAA GGAGCACCGGATGCTGACCACAACTCGTTACAGCTGAACTGACCTCTCTTTCTTACCTT
+1	PheArgSerGlyGlyAspValAspHisSerThrLeuValThrLeuPheLysLeuLeuGly
601	TTTCGCTCTGGAGGGGATGTGGACCAACAGTACTTTAGTCAACCTCTTCAAGCTTTTGGGC AAAGCGAGACCTCCCTACACCTGGTGTATGAGATCTGTGGAGAGAGTTGGAAGGCGG

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Fig. 12B

CASPASE 2

```

+1 TyrAspValHisValLeuCysAspGlnThrAlaGlnGluMetGlnGluLysLeuGlnAsn
-----
661 TATGACGTCCATGTTCTATGTGACCAGACTGCACAGGAAATGCAAGAGAACTGCAGAAT
    ATACTGCAGGTACAAGATACACTGGTCTGACGTGTCCTTTACGTTCTCTTTGACGTCTTA
-----
+1 PheAlaGlnLeuProAlaHisArgValThrAspSerCysIleValAlaLeuLeuSerHis
-----
721 TTTGCACAGTTACCTGCACACCGAGTCACGGACTCCTGCATCGTGGCACTCCTCTCGCAT
    AAACGTGTCAATGGACGTGTGGCTCAGTGCCTGAGGACGTAGCACCGTGAGGAGAGCGTA
-----
+1 GlyValGluGlyAlaIleTyrGlyValAspGlyLysLeuLeuGlnLeuGlnGluValPhe
-----
781 GGTGTGGAGGGCGCCATCTATGGTGTGGATGGGAACTGCTCCAGCTCCAAGAGGTTTTT
    CCACACCTCCCGCGGTAGATACCACACCTACCCTTTGACGAGGTGAGGTTCTCCAAAAA
-----
+1 GlnLeuPheAspAsnAlaAsnCysProSerLeuGlnAsnLysProLysMetPhePheIle
-----
841 CAGCTCTTTGACAACGCCAACTGCCCAAGCCTACAGAACAACCAAAAATGTTCTTCATC
    GTCGAGAACTGTTGCGGTTGACGGGTTGCGATGTCTTGTGTTTGTGTTTACAAGAAGTAG
-----
+1 GlnAlaCysArgGlyAspGluThrAspArgGlyValAspGlnGlnAspGlyLysAsnHis
-----
901 CAGGCCTGCCGTGGAGATGAGACTGATCGTGGGGTTGACCAACAAGATGGAAAGAACCAC
    GTCCGGACGGCACCTCTACTCTGACTAGCACCCCAACTGGTTGTTCTACCTTTCTTGGTG
-----
+1 AlaGlySerProGlyCysGluGluSerAspAlaGlyLysGluLysLeuProLysMetArg
-----
961 GCAGGATCCCTGGGTGCGAGGAGAGTGATGCCGGTAAAGAAAAGTTGCCGAAGATGAGA
    CGTCCTAGGGGACCCACGCTCCTCTCACTACGGCCATTTCTTTTCAACGGCTTCTACTCT
-----
+1 LeuProThrArgSerAspMetIleCysGlyTyrAlaCysLeuLysGlyThrAlaAlaMet
-----
1021 CTGCCCACGCGCTCAGACATGATATGCGGCTATGCCTGCCTCAAAGGGACTGCCGCCATG
    GACGGGTGCGCGAGTCTGTACTATACGCCGATACGGACGGAGTTTCCCTGACGGCGGTAC
-----
+1 ArgAsnThrLysArgGlySerTrpTyrIleGluAlaLeuAlaGlnValPheSerGluArg
-----
1081 CGGAACACCAAACGAGGTTCCCTGGTACATCGAGGCTCTTGCTCAAGTGTTTTCTGAGCGG
    GCCTTGTTGGTTTGCTCCAAGGACCATGTAGCTCCGAGAACGAGTTCACAAAAGACTCGCC
-----
+1 AlaCysAspMetHisValAlaAspMetLeuValLysValAsnAlaLeuIleLysAspArg
-----
1141 GCTTGTGATATGCACGTGGCCGACATGCTGGTTAAGGTGAACGCCTTATCAAGGATCGG
    CGAACACTATACGTGCACCGGCTGTACGACCAATTCCACTTGCGTGAATAGTTCTTAGCC
-----
+1 GluGlyTyrAlaProGlyThrGluPheHisArgCysLysGluMetSerGluTyrCysSer
-----
1201 GAAGGTTATGCTCCTGGCACAGAAATCCACCGGTGCAAGGAAATGTCTGAATACTGCAGC
    CTTCCAATACGAGGACCGTGTCTTAAGGTGGCCACGTTCTTTTACAGACTTATGACGTGG
-----
+1 ThrLeuCysArgHisLeuTyrLeuPheProGlyHisProProThr
-----
1261 ACTCTGTGCCGCCACCTCTACCTGTTCCCGAGGACACCCCTCCACATGA
    TGAGACACGGCGGTGGAGATGGACAAGGGTCTGTGGGAGGGTGTACT

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Fig. 13A

CASPASE 3

```

+1 MetGluAsnThrGluAsnSerValAspSerLysSerIleLysAsnLeuGluProLysIle
]-----
1  ATGGAGAACACTGAAAACTCAGTGGATTCAAAATCCATTAAAAATTTGGAACCAAAGATC
TACCTCTTGTGACTTTTGAGTCACCTAAGTTTTAGGTAATTTTTAAACCTTGGTTTTCTAG
-----
+1 IleHisGlySerGluSerMetAspSerGlyIleSerLeuAspAsnSerTyrLysMetAsp
-----
61 ATACATGGAAGCGAATCAATGGACTCTGGAATATCCCTGGACAACAGTTATAAAATGGAT
TATGTACCTTCGCTTAGTTACCTGAGACCTTATAGGGACCTGTTGTCAATATTTTACCTA
-----
+1 TyrProGluMetGlyLeuCysIleIleIleAsnAsnLysAsnPheHisLysSerThrGly
-----
121 TATCCTGAGATGGGTTTATGTATAATAATTAATAATAAGAATTTTCATAAAAGCACTGGA
ATAGGACTCTACCCAAATACATAATTATTAATTATTATTCTTAAAAGTATTTTCGTGACCT
-----
+1 MetThrSerArgSerGlyThrAspValAspAlaAlaAsnLeuArgGluThrPheArgAsn
-----
181 ATGACATCTCGGTCTGGTACAGATGTCGATGCAGCAAACCTCAGGGAAACATTCAGAAAC
TACTGTAGAGCCAGACCATGTCTACAGCTACGTCGTTTGGAGTCCCTTTGTAAGTCTTTG
-----
+1 LeuLysTyrGluValArgAsnLysAsnAspLeuThrArgGluGluIleValGluLeuMet
-----
241 TTGAAATATGAAGTCAGGAATAAAAATGATCTTACACGTGAAGAAATTGTGGAATTGATG
AACTTTTATACTTCAGTCCTTATTTTTTACTAGAATGTGCACTTCTTTAACACCTTAACAC
-----
+1 ArgAspValSerLysGluAspHisSerLysArgSerSerPheValCysValLeuLeuSer
-----
301 CGTGATGTTTCTAAAGAAGATCACAGCAAAAGGAGCAGTTTTGTTGTGTGCTTCTGAGC
GCACTACAAAGATTTCTTCTAGTGTCGTTTTCTCGTCAAAACAAACACACGAAGACTCG
-----
+1 HisGlyGluGluGlyIleIlePheGlyThrAsnGlyProValAspLeuLysLysIleThr
-----
361 CATGGTGAAGAAGGAATAATTTTTTGAACAAATGGACCTGTTGACCTGAAAAAATAACA
GTACCACTTCTTCCTTATTAAAAACCTTGTTTACCTGGACAACCTGGACTTTTTTTATTGT
-----
+1 AsnPhePheArgGlyAspArgCysArgSerLeuThrGlyLysProLysLeuPheIleIle
-----
421 AACTTTTTTCAGAGGGGATCGTTGTAGAAGTCTAACTGGAAAACCCAAACTTTTCATTATT
TTGAAAAAGTCTCCCCTAGCAACATCTTCAGATTGACCTTTTGGGTTTGAAAAGTAATAA
-----
+1 GlnAlaCysArgGlyThrGluLeuAspCysGlyIleGluThrAspSerGlyValAspAsp
-----
481 CAGGCCTGCCGTGGTACAGAACTGGACTGTGGCATTGAGACAGACAGTGGTGTGATGAT
GTCCGGACGGCACCATGTCTTGACCTGACACCGTAACCTCTGTCTGTACCCACAACACTACTA
-----
+1 AspMetAlaCysHisLysIleProValAspAlaAspPheLeuTyrAlaTyrSerThrAla
-----
541 GACATGGCGTGTGCATAAAATACCAGTGGATGCGCACTTCTTGTATGCATACTCCACAGCA
CTGTACCGCACAGTATTTTATGGTCACTACGGCTGAAGAACATACGTATGAGGTGTGGT
-----
+1 ProGlyTyrTyrSerTrpArgAsnSerLysAspGlySerTrpPheIleTrpSerLeuLys
-----
601 CCTGGTTATTATTCTTGGCGAAATTCAAAGGATGGCTCCTGCTTCATCCAGTCGCTTTGT
GGACCAATAATAAGAACCTCTTTAAGTTTCTACCGGGGALCAAGTAGGTCTAGCGAAACA

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Fig. 13B

CASPASE 3

```
+1 AlaMetLeuLysGlnTyrAlaAspLysLeuGluPheMetHisIleLeuThrArgValAsn
-----
661 GCCATGCTGAAACAGTATGCCGACAAGCTTGAATTTATGCACATTCTTACCCGGGTAAAC
    CGGTACGACTTTGTCATACGGCTGTTTGAAGTTAAATACGTGTAAGAATGGGCCCAATTG
-----
+1 ArgLysValAlaThrGluPheGluSerPheSerPheAspAlaThrPheHisAlaLysLys
-----
721 CGAAAGGTGGCAACAGAAATTTGAGTCCTTTTCCTTTGACGCTACTTTTCATGCAAAGAAA
    GCTTTCCACCGTTGTCTTAACTCAGGAAAAGGAAACTGCGATGAAAAGTACGTTTCTTT
-----
+1 GlnIleProCysIleValSerMetLeuThrLysGluLeuTyrPheTyrHis
----->
781 CAGATTCCATGTATTGTTTCCATGCTCACAAAAGAACTCTATTTTATCACTAAN
    GTCTAAGGTACATAACAAAGGTACGAGTGTTTTCTTGAGATAAAAATAGTGATTN
-----
```

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Fig. 14A

CASPASE 4

```

+1 MetAlaGluGlyAsnHisArgLysLysProLeuLysValLeuGluSerLeuGlyLysAsp
1 ATGGCAGAAGGCAACCACAGAAAAAGCCACTTAAGGTGTTGGAATCCCTGGGCAAAGAT
TACCGTCTTCCGTTGGTGTCTTTTTTCGGTGAATTCCACAACCTTAGGGACCCGTTTCTA
+1 PheLeuThrGlyValLeuAspAsnLeuValGluGlnAsnValLeuAsnTrpLysGluGlu
61 TTCCTCACTGGTGTGTTTGGATAACTTGGTGGACAAAATGTACTGAACTGGAAGGAAGAG
AAGGAGTGACCACAAAACCTATTGAACCACCTTGTTTTACATGACTTGACCTTCCTTCTC
+1 GluLysLysLysTyrTyrAspAlaLysThrGluAspLysValArgValMetAlaAspSer
121 GAAAAAAGAAATATTACGATGCTAAACTGAAGACAAAGTTCGGGTCATGGCAGACTCT
CTTTTTTCTTTATAATGCTACGATTTTGACTTCTGTTTCAAGCCCAGTACCGTCTGAGA
+1 MetGlnGluLysGlnArgMetAlaGlyGlnMetLeuLeuGlnThrPhePheAsnIleAsp
181 ATGCAAGAGAAGCAACGTATGGCAGGACAAATGCTTCTTCAAACCTTTTTTAACATAGAC
TACGTTCTCTTCGTTGCATACCGTCTGTTTACGAAGAAGTTTGGAAAAAATTGTATCTG
+1 GlnIleSerProAsnLysLysAlaHisProAsnMetGluAlaGlyProProGluSerGly
241 CAAATATCCCCCAATAAAAAAGCTCATCCGAATATGGAGGCTGGACCACCTGAGTCAGGA
GTTTATAGGGGGTTATTTTTTCGAGTAGGCTTATACCTCCGACCTGGTGGACTCAGTCCT
+1 GluSerThrAspAlaLeuLysLeuCysProHisGluGluPheLeuArgLeuCysLysGlu
301 GAATCTACAGATGCCCTCAAGCTTTGTCTCATGAAGAATTCCTGAGACTATGTAAAGAA
CTTAGATGTCTACGGGAGTTCCGAAACAGGAGTACTTCTTAAGGACTCTGATACATTTCTT
+1 ArgAlaGluGluIleTyrProIleLysGluArgAsnAsnArgThrArgLeuAlaLeuIle
361 AGAGCTGAAGAGATCTATCCAATAAAGGAGAGAAACAACCGCACACGCCTGGCTCTCATC
TCTCGACTTCTCTAGATAGGTTATTTCTCTCTTTGTTGGCGTGTGGGACCGAGAGTAG
+1 IleCysAsnThrGluPheAspHisLeuProProArgAsnGlyAlaAspPheAspIleThr
421 ATATGCAATACAGAGTTTGACCATCTGCCTCCGAGGAATGGAGCTGACTTTGACATCACA
TATACGTTATGTCTCAAACCTGGTAGACGGAGGCTCCTTACCTCGACTGAAACTGTAGTGT
+1 GlyMetLysGluLeuLeuGluGlyLeuAspTyrSerValAspValGluGluAsnLeuThr
481 GGGATGAAGGAGCTACTTCAGGGTCTGGACTATAGTGTAGATGTAGAGAGAATCTGACA
CCCTACTTCCTCGATGAACCTCCAGACCTGATATCACATCTACATTTCTCTTAGACTGT
+1 AlaArgAspMetGluSerAlaLeuArgAlaPheAlaThrArgProGluHisLysSerSer
541 GCCAGGGATATGGAGTCAGGCTGAGGGGCAATTCCTACCAGAGCGAATGACCAAGTCCTCT
CGGTCCCTATACCTCAGTCGAGATTTCCGTAACGATGGTCTGGTCTGTGTTCAGGAGA
+1 AspSerThrPheLeuValLeuMetIleHisGlyIleLeuGluGluIleLeuGlyThrVal
601 GACAGCACATTCTTGTGACTTATGTCTCATGGCATCCTGGAGGGAAATCTGGGAACTGTG
CTGTCTGTGAAGAACCATGATACGATGATAGCTAGGACCTGGTCTTACAGCCTTGACAC

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Fig. 14B

CASPASE 4

```

+1 HisAspGluLysLysProAspValLeuLeuTyrAspThrIlePheGlnIlePheAsnAsn
-----
661 CATGATGAGAAAAAACCAGATGTGCTGCTTTATGACACCATCTTCCAGATATTCAACAAC
GTACTACTCTTTTTTGGTCTACACGACGAAATACTGTGGTAGAAGGTCTATAAGTTGTTG
+1 ArgAsnCysLeuSerLeuLysAspLysProLysValIleIleValGlnAlaCysArgGly
-----
721 CGCAACTGCCTCAGTCTGAAGGACAAACCCAAGGTCATCATTGTCCAGGCCTGCAGAGGT
GCGTTGACGGAGTCAGACTTCCTGTTTGGGTTCCAGTAGTAACAGGTCCGGACGTCTCCA
+1 AlaAsnArgGlyGluLeuTrpValArgAspSerProAlaSerLeuGluValAlaSerSer
-----
781 GCAAACCGTGGGGAACGTGTTGGGTCAGAGACTCTCCAGCATCCTTGGAAGTGGCCTCTTCA
CGTTTGGCACCCCTTGACACCCAGTCTCTGAGAGGTCGTAGGAACCTTCACCGGAGAAGT
+1 GlnSerSerGluAsnLeuGluGluAspAlaValTyrLysThrHisValGluLysAspPhe
-----
841 CAGTCATCTGAGAACCTGGAGGAAGATGCTGTTTACAAGACCCACGTGGAGAAGGACTTC
GTCAGTAGACTCTTGGACCTCCTTCTACGACAAATGTTCTGGGTGCACCTCTTCCTGAAG
+1 IleAlaPheCysSerSerThrProHisAsnValSerTrpArgAspSerThrMetGlySer
-----
901 ATTGCTTTCTGCTCTTCAACGCCACACAACGTGTCTCTGGAGAGACAGCACAATGGGCTCT
TAACGAAAGACGAGAAGTTGCGGTGTGTTGCACAGGACCTCTCTGTCGTGTTACCCGAGA
+1 IlePheIleThrGlnLeuIleThrCysPheGlnLysTyrSerTrpCysCysHisLeuGlu
-----
961 ATCTTCATCACACAACCTCATCACATGCTTCCAGAAATATTCTTGGTGCTGCCACCTAGAG
TAGAAGTAGTGTTGAGTAGTGACGAAGGTCTTTATAAGAACCACGACGGTGGATCTC
+1 GluValPheArgLysValGlnGlnSerPheGluThrProArgAlaLysAlaGlnMetPro
-----
1021 GAAGTATTTCCGAAGGTACAGCAATCATTTGAAACTCCAAGGGCCAAAGCTCAAATGCCC
CTTCATAAAGCCTTCCATGTCGTTAGTAACTTTGAGGTTCCCGGTTTCGAGTTTACGGG
+1 ThrIleGluArgLeuSerMetThrArgTyrPheTyrLeuPheProGlyAsn
----->
1081 ACCATAGAACGACTGTCCATGACAAGATATTTCTACCTCTTTCCTGGCAATTGA
TGGTATCTTGCTGACAGGTACTGTTCTATAAAGATGGAGAAAGGACCGTTAACT

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Fig. 15A

CASPASE 5

```

+1 MetPheLysGlyIleLeuGlnSerGlyLeuAspAsnPheValIleAsnHisMetLeuLys
1  ATGTTCAAAGGTATCCTTCAGAGTGGATTGGATAACTTCGTGATAAACCACATGCTAAAG
   TACAAGTTTCCATAGGAAGTCTCACCTAACCTATTGAAGCACTATTTGGTGTACGATTTCT
+1 AsnAsnValAlaGlyGlnThrSerIleGlnThrLeuValProAsnThrAspGlnLysSer
61  AACAACGTGGCTGGACAAACATCTATCCAGACCCTAGTACCTAATACGGATCAAAAAGTCG
   TTGTTGCACCGACCTGTTTGTAGATAGGTCTGGGATCATGGATTATGCCTAGTTTTTCAGC
+1 ThrSerValLysLysAspAsnHisLysLysLysThrValLysMetLeuGluTyrLeuGly
121  ACCAGTGTAAGAAAAAGACAACCACAAAAAAGACAGTTAAGATGTTGGAATACCTGGGC
   TGGTCACATTTTTTCTGTTGGTGTTTTTTTTTTGTCAATTCTACAACCTTATGGACCCG
+1 LysAspValLeuHisGlyValPheAsnTyrLeuAlaLysHisAspValLeuThrLeuLys
181  AAAGATGTTCTTCATGGTGTTTTTTAATTATTTGGCAAAACACGATGTTCTGACATTGAAG
   TTTCTACAAGAAGTACCACAAAAATTAATAAACCGTTTTGTGCTACAAGACTGTAACCTC
+1 GluGluGluLysLysLysTyrTyrAspAlaLysIleGluAspLysAlaLeuIleLeuVal
241  GAAGAGGAAAAAGAAAAAATATTATGATGCCAAAATTGAAGACAAGGCCCTGATCTTGGTA
   CTTCTCCTTTTTCTTTTTTATAATACTACGGTTTTAACTTCTGTTCCGGGACTAGAACCAT
+1 AspSerLeuArgLysAsnArgValAlaHisGlnMetPheThrGlnThrLeuLeuAsnMet
301  GACTCTTTGCGAAAGAATCGCGTGGCTCATCAATGTTTACCCAAACACTTCTCAATATG
   CTGAGAAACGCTTTCTTAGCGCACCGAGTAGTTTACAAATGGGTTTGTGAAGAGTTATAC
+1 AspGlnLysIleThrSerValLysProLeuLeuGlnIleGluAlaGlyProProGluSer
361  SACCAAAAGATCACCAGTGTAACCTCTTCTGCAAATCGAGGCTGGACCACCTGAGTCA
   CTGGTTTTCTAGTGGTCACATTTTGGAGAAGACGTTTAGCTCCGACCTGGTGGACTCAGT
+1 AlaGluSerThrAsnIleLeuLysLeuCysProArgGluGluPheLeuArgLeuCysLys
421  GCAGAATCTACAAATATACTCAAACCTTTGTCTCGTGAAGAATTCCTGAGACTGTGTAAA
   CGTCTTAGATGTTTATATGAGTTTGAACAGGAGCACTTCTTAAGGACTCTGACACATTT
+1 LysAsnHisAspGluIleTyrProIleLysLysArgGluAspArgArgArgLeuAlaLeu
481  AAAAAATCATGATGAGATCTATCCAATAAAAAAGAGAGAGGACCGCAGACGCCTGGCTCTC
   TTTTGTAGTACTACTCTAGATAGGTTATTTTTTCTCTCTCCTGGCGTCTGGCGACCGAGAG
+1 IleIleCysAsnThrLysPheAspHisLeuProAlaArgAsnGlyAlaHisTyrAspIle
541  ATCATATGCAATACAAAGTTTGTATCACTTGCCTGCAAGGAATGGGGCTCACTATGACATC
   TAGTATACGTTATGTTTCAAACTAGTGGACGGACGTTCCCTTACCCGAGTGTACTGTAG
+1 ValGlyMetLysArgLeuLeuGlnGlyLeuGlyTyrThrValValAspGluLysAsnLeu
601  GTGGGGATGAAAAGGCTGCTTCAAGGCTGGGCTACACTGTGCTTCAAGTAAAGAAATCTC
   CACCCCTACTTTCCGACGAAATCCGACCCGATGTGACACAACTCTCTTTCTTAGAG

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Fig. 15B

CASPASE 5

```

-1 ThrAlaArgAspMetGluSerValLeuArgAlaPheAlaAlaArgProGluHisLysSer
-----
661 ACAGCCAGGGATATGGAGTCAGTGCTGAGGGCATTGCTGCCAGACCAGAGCACAAGTCC
TGTCCGGTCCCTATACCTCAGTCACGACTCCCGTAAACGACGGTCTGGTCTCGTGTTTCAGG
-----
-1 SerAspSerThrPheLeuValLeuMetSerHisGlyIleLeuGluGlyIleCysGlyThr
-----
721 TCTGACAGCACGTTCTTGGTACTCATGTCTCATGGCATCCTAGAGGGAATCTGCGGAAC
TACACTGTCGTGCAAGAACCATGAGTACAGAGTACCGTAGGATCTCCCTTAGACGCCTTGA
-----
+1 AlaHisLysLysLysLysProAspValLeuLeuTyrAspThrIlePheGlnIlePheAsn
-----
781 GCGCATAAAAAGAAAAAACCGGATGTGCTGCTTTATGACACCATCTTCCAGATATTCAAC
CGCGTATTTTTCTTTTTTGGCCTACACGACGAAATACTGTGGTAGAAGGTCTATAAGTTG
-----
-1 AsnArgAsnCysLeuSerLeuLysAspLysProLysValIleIleValGlnAlaCysArg
-----
841 AACCGCAACTGCCTCAGTCTAAAGGACAAACCCAAGGTCATCATTGTCCAGGCCTGCAGA
TTGGCGTTGACGGAGTCAGATTTCTGTTTGGGTTCAGTAGTAACAGGTCCGGACGTCT
-----
+1 GlyGluLysHisGlyGluLeuTrpValArgAspSerProAlaSerLeuAlaValIleSer
-----
901 GGTGAAAAACATGGGGAACCTCTGGGTCAGAGACTCTCCAGCATCCTTGGCAGTCATCTCT
CCACTTTTTGTACCCCTTGAGACCCAGTCTCTGAGAGGTCGTAGGAACCGTCAGTAGAGA
-----
+1 SerGlnSerSerGluAsnLeuGluAlaAspSerValCysLysIleHisGluGluLysAsp
-----
961 TCACAGTCATCTGAGAACCTGGAGGCAGATTCTGTTTGCAAGATCCACGAGGAGAAGGAC
AGTGTCAGTAGACTCTTGGACCTCCGTCTAAGACAAACGTTCTAGGTGCTCCTCTTCCTG
-----
-1 PheIleAlaPheCysSerSerThrProHisAsnValSerTrpArgAspArgThrArgGly
-----
1021 TTCATTGCTTTCTGTTCTTCAACACCACATAACGTGTCCTGGAGAGACCGCACAAAGGGGC
AAGTAACGAAAGACAAGAAGTTGTGGTGTATTGCACAGGACCTCTCTGGCGTGTTCCTCCG
-----
+1 SerIlePheIleThrGluLeuIleThrCysPheGlnLysTyrSerCysCysCysHisLeu
-----
1081 TCCATCTTCATTACGGAACCTCATCACATGCTTCCAGAAATATTCTTGCTGCTGCCACCTA
AGGTAGAAGTAATGCCTTGAGTAGTGTACGAAGGTCTTTATAAGAACGACGACGGTGGAT
-----
+1 MetGluIlePheArgLysValGlnLysSerPheGluValProGlnAlaLysAlaGlnMet
-----
1141 ATGGAAATATTTGGGAAGGTACAGAAATCATTTGAAGTTCACAGGCTAAAGCCAGATG
TACCTTTATAAAGCCTTCCATGTCTTTAGTAAACTTCAAGGTGTCCGATTTCCGGGTCTAC
-----
-1 ProThrIleGluArgAlaThrLeuThrArgAspPheTyrLeuPheProGlyAsn
-----
1201 CCCACCATAGAACGAGCAACCTTGACAAGAGATTCTACCTCTTTCTCTGGCAATTGAA
GGGTGGTATCTTCTCTCTGGAACTGTTCTCTAAGATGGAGAAAGGACCGTAACTN

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Fig. 16A

CASPASE 6

+1	MetSerSerAlaSerGlyLeuArgArgGlyHisProAlaGlyGlyGluGluAsnMetThr
1	ATGAGCTCGGCCTCGGGGCTCCGCAGGGGGCACCCGGCAGGTGGGGAAGAAAACATGACA TACTCGAGCCGGAGCCCCGAGGCGTCCCCGTGGGCGGTCCACCCCTTCTTTTGTACTGT
+1	GluThrAspAlaPheTyrLysArgGluMetPheAspProAlaGluLysTyrLysMetAsp
61	GAAACAGATGCCTTCTATAAAAGAGAAATGTTTGTATCCGGCAGAAAAGTACAAAATGGAC CTTTGTCTACGGAAGATATTTCTCTTTACAACTAGGCCGTCTTTTCATGTTTTACCTG
+1	HisArgArgArgGlyIleAlaLeuIlePheAsnHisGluArgPhePheTrpHisLeuThr
121	CACAGGAGGAGAGGAATTGCTTTAATCTTCAATCATGAGAGGTTCTTTTGGCACTTAACA GTGTCTCTCTCTCTTAACGAAATTAGAAGTTAGTACTCTCCAAGAAAACCGTGAATTGT
+1	LeuProGluArgArgGlyThrCysAlaAspArgAspAsnLeuThrArgArgPheSerAsp
181	CTGCCAGAAAGGCGGGGCACCTGCGCAGATAGAGACAATCTTACCCGCAGGTTTTTCAGAT GACGGTCTTTCCGCCCGTGGACGCGTCTATCTCTGTTAGAATGGGCGTCCAAAAGTCTA
+1	LeuGlyPheGluValLysCysPheAsnAspLeuLysAlaGluGluLeuLeuLysIle
241	CTAGGATTTGAAGTGAAATGCTTTAATGATCTTAAAGCAGAAGAAGTACTGCTCAAAATT GATCCTAAACTTCACTTTACGAAATTACTAGAATTTTCGTCTTCTTGATGACGAGTTTTAA
+1	HisGluValSerThrValSerHisAlaAspAlaAspCysPheValCysValPheLeuSer
301	CATGAGGTGTCAACTGTTAGCCACGCAGATGCCGATTGCTTTGTGTGTGCTTTCCTGAGC GTACTCCACAGTTGACAATCGGTGCGTCTACGGCTAACGAAACACACACAGAAGGACTCG
+1	HisGlyGluGlyAsnHisIleTyrAlaTyrAspAlaLysIleGluIleGlnThrLeuThr
361	CATGGCGAAGGCAATCACATTTATGCATATGATGCTAAATCGAAATTCAGACATTAACT GTACCGCTTCCGTTAGTGTAATACGTATACTACGATTTTAGCTTTAAGTCTGTAATTGA
+1	GlyLeuPheLysGlyAspLysCysHisSerLeuValGlyLysProLysIlePheIleIle
421	GGCTTGTTCAAAGGAGACAAGTGTACAGCCTGGTTGGAAAACCAAGATATTTATCATC CCGAACAAGTTTCTCTGTTTCACAGTGTGCGACCAACCTTTTGGGTTCTATAAATAGTAG
+1	GlnAlaCysArgGlyAsnGlnHisAspValProValIleProLeuAspValValAspAsn
481	CAGGCATGTGGGGAAACCAGCACGATGTGCCAGTCATTCTTTGGATGTAGTAGATAAT GTCCGTACAGCCCCTTTGGTGGTGCTACACGGTCAGTAAGGAAACCTACATCATCTATTA
+1	GlnThrGluLysLeuAspThrAsnIleThrGluValAspAlaAlaSerValTyrThrLeu
541	CAGACAGAGAAGTTGGACACCAACATAAAGTGGGTGGATGCAGCCTCCGTTTACACGCTG GTCTGTCTCTTCAACCTGTGGTTGATTAAGTCCACCTACGTGGGAGGCAATGTGGAC
+1	ProAlaGlyAlaAspPheLeuMetCysTyrSerValAlaGluGlyTyrTyrSerHisArg
601	CCTGCTGGAGCTGACTTCTCATGTGTTACTCTGTTGCAGAAAGATATTATTCTACCCGG GGACGACCTCGACTGAGGAGTACACAAATGAGACAAATGCTTCTATATTAAGAGTGGCC

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Fig. 16B

CASPASE 6

```
+1 GluThrValAsnGlySerTrpTyrIleGlnAspLeuCysGluMetLeuGlyLysTyrGly
-----
661 GAAACTGTGAACGGCTCATGGTACATTCAAGATTTGTGTGAGATGTTGGGAAAATATGGC
CTTTGACACTTGCCGAGTACCATGTAAAGTTCTAAACACACTCTACAACCCTTTTATACCG
-----
+1 SerSerLeuGluPheThrGluLeuLeuThrLeuValAsnArgLysValSerGlnArgArg
-----
721 TCCTCCTTAGAGTTTCACAGAACTCCTCACACTGGTGAACAGGAAAGTTTCTCAGCGCCGA
AGGAGGAATCTCAAGTGTCTTGAGGAGTGTGACCACTTGTCCTTTCAAAGAGTCGCGGCT
-----
+1 ValAspPheCysLysAspProSerAlaIleGlyLysLysGlnValProCysPheAlaSer
-----
781 GTGGACTTTTGCAAAGACCCAAGTGCAATTGGAAAGAAGCAGGTTCCCTGTTTTGCCTCA
CACCTGAAAACGTTTCTGGGTTACGTTAACCTTTCTTCGTCCAAGGGACAAAACGGAGT
-----
+1 MetLeuThrLysLysLeuHisPhePheProLysSerAsn
----->
841 ATGCTAACTAAAAAGCTGCATTTCTTTCCAAAATCTAATTAAN
TACGATTGATTTTTTCGACGTAAAGAAAGGTTTTAGATTAATTN
-----
```

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Fig. 17A

CASPASE 7

```

+1 MetAlaAspAspGlnGlyCysIleGluGluGlnGlyValGluAspSerAlaAsnGluAsp
]-----
1  ATGGCAGATGATCAGGGCTGTATTGAAGAGCAGGGGGTTGAGGATTCAGCAAATGAAGAT
   TACCGTCTACTAGTCCCGACATAACTTCTCGTCCCCCAACTCCTAAGTCGTTTACTTCTA
+1 SerValAspAlaLysProAspArgSerSerPheValProSerLeuPheSerLysLysLys
-----
61 TCAGTGGATGCTAAGCCAGACCGGTCTCGTTTGTACCGTCCCTCTTCAGTAAGAAGAAG
   AGTCACCTACGATTTCGGTCTGGCCAGGAGCAAACATGGCAGGGAGAAGTCATTCTTCTTC
+1 LysAsnValThrMetArgSerIleLysThrThrArgAspArgValProThrTyrGlnTyr
-----
121 AAAAATGTCACCATGCGATCCATCAAGACCACCCGGGACCGAGTGCCTACATATCAGTAC
   TTTTACAGTGGTACGCTAGGTAGTTCTGGTGGGCCCTGGCTCACGGATGTATAGTCATG
+1 AsnMetAsnPheGluLysLeuGlyLysCysIleIleIleAsnAsnLysAsnPheAspLys
-----
181 AACATGAATTTTGAAGCTGGGCAAATGCATCATAATAACAACAAGAACTTTGATAAA
   TTGTACTTAAACCTTTTCGACCCGTTTACGTAGTATTATTTGTTGTTCTTGAAACTATTT
+1 ValThrGlyMetGlyValArgAsnGlyThrAspLysAspAlaGluAlaLeuPheLysCys
-----
241 GTGACAGGTATGGGCGTTTCGAAACGGAACAGACAAAGATGCCGAGGCGCTCTTCAAGTGC
   CACTGTCCATACCCGCAAGCTTTGCCTTGTCTGTTTCTACGGCTCCGCGAGAAGTTCACG
+1 PheArgSerLeuGlyPheAspValIleValTyrAsnAspCysSerCysAlaLysMetGln
-----
301 TTCCGAAGCCTGGGTTTTGACGTGATTGTCTATAATGACTGCTCTTGTGCCAAGATGCAA
   AAGGCTTCGGACCCAAAACCTGCACCTAACAGATATTACTGACGAGAACACGGTTCTACGTT
+1 AspLeuLeuLysLysAlaSerGluGluAspHisThrAsnAlaAlaCysPheAlaCysIle
-----
361 GATCTGCTTAAAAAAGCTTCTGAAGAGGACCATACAAATGCCGCCTGCTTCGCCTGCATC
   CTAGACGAATTTTTTCGAAGACTTCTCCTGGTATGTTTACGGCGGACGAAGCGGACGTAG
+1 LeuLeuSerHisGlyGluGluAsnValIleTyrGlyLysAspGlyValThrProIleLys
-----
421 CTCTTAAGCCATGGAGAAGAAAATGTAATTTATGGGAAAGATGGTGTACACCAATAAAG
   GAGAATTCGGTACCTCTTCTTTTACATTAAATACCCTTTCTACCACAGTGTGGTTATTTT
+1 AspLeuThrAlaHisPheArgGlyAspArgCysLysThrLeuLeuGluLysProLysLeu
-----
481 GATTTGACAGCCCACTTTAGGGGGGATAGATGC4AAACCCTTTTAGAGAAACCCAAACTC
   CTAAACTGTGGGGTGAAATCCCCCTATCTACGTTTGGGAAAATCTCTTTGGGTTTGAG
+1 PhePheIleGlnAlaCysArgGlyThrGluLeuAspAspGlyIleGlnAlaAspSerGly
-----
541 TTCTTCATTTCAGGCTTGCCGAGGGACCGAGCTTGATGATGGCATCCAGGCCGACTCGGGG
   AAGAAGTAAGTCCGAACGGGCTCCCTGGCTCGAATCTACCGGTAGGTCCGGCTGAGCCCG
+1 ProIleAsnAspThrAspAlaAsnProArgIleLeuIlePheValGluAlaAspProLeu
-----
601 CCCATCAATGACACAGATGCTAATCCTCGATACAAATCCAGTGGGAAGCTGACTTCTCTC
   GGGTAGCTTACTGTCTCTAGGATTAGGAGCTATCTTTAGGGTCACCTTCGACTGAAAGAA

```

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Fig. 17B

CASPASE 7

```
+1 PheAlaTyrSerThrValProGlyTyrTyrSerTrpArgSerProGlyArgGlySerTrp
-----
661 TTCGCCTATTCCACGGTTCCAGGCTATTACTCGTGGAGGAGCCCAGGAAGAGGCTCCTGG
AAGCGGATAAGGTGCCAAGGTCCGATAATGAGCACCTCCTCGGGTCCTTCTCCGAGGACC
-----
+1 PheValGlnAlaLeuCysSerIleLeuGluGluHisGlyLysAspLeuGluIleMetGln
-----
721 TTTGTGCAAGCCCTCTGCTCCATCCTGGAGGAGCACGGAAAAGACCTGGAAATCATGCAG
AAACACGTTCCGGGAGACGAGGTAGGACCTCCTCGTGCCTTTTCTGGACCTTTAGTACGTC
-----
+1 IleLeuThrArgValAsnAspArgValAlaArgHisPheGluSerGlnSerAspAspPro
-----
781 ATCCTCACCAGGGTGAATGACAGAGTTGCCAGGCACTTTGAGTCTCAGTCTGATGACCCA
TAGGAGTGGTCCCCTTACTGTCTCAACGGTCCGTGAACTCAGAGTCAGACTACTGGGT
-----
+1 HisPheHisGluLysLysGlnIleProCysValValSerMetLeuThrLysGluLeuTyr
-----
841 CACTTCCATGAGAAGAAGCAGATCCCCTGTGTGGTCTCCATGCTCACCAAGGAACTCTAC
GTGAAGGTACTCTTCTTCGTCTAGGGGACACACCAGAGGTACGAGTGGTTCCTTGAGATG
-----
+1 PheSerGln
----->
901 TTCAGTCAATAGN
AAGTCAGTTATCN
```

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Fig. 18A

CASPASE 3

```

+1 MetAspPheSerArgAsnLeuTyrAspIleGlyGluGlnLeuAspSerGluAspLeuAla
]-----
1  ATGGACTTCAGCAGAAATCTTTATGATATTGGGGAACAACCTGGACAGTGAAGATCTGGCC
TACCTGAAGTCGTCTTTAGAAATACTATAACCCCTTGTTGACCTGTCACCTTAGACCGG
-----
+1 SerLeuLysPheLeuSerLeuAspTyrIleProGlnArgLysGlnGluProIleLysAsp
-----
61  TCCCTCAAGTTCTTGAGCCTGGACTACATTCCGCAAAGGAAGCAAGAACCCATCAAGGAT
AGGGAGTTCAAGGACTCGGACCTGATGTAAGGCGTTTCCTTCGTTCTTGGGTAGTTCCTA
-----
+1 AlaLeuMetLeuPheGlnArgLeuGlnGluLysArgMetLeuGluGluSerAsnLeuSer
-----
121  GCCTTGATGTTATTCCAGAGACTCCAGGAAAAGAGAATGTTGGAGGAAAGCAATCTGTCC
CGGAAC TACAATAAGGTCTCTGAGGTCCTTTTCTCTTACAACCTCCTTTTCGTTAGACAGG
-----
+1 PheLeuLysGluLeuLeuPheArgIleAsnArgLeuAspLeuLeuIleThrTyrLeuAsn
-----
181  TTCCTGAAGGAGCTGCTCTTCCGAATTAATAGACTGGATTTGCTGATTACCTACCTAAAC
AAGGACTTCCTCGACGAGAAGGCTTAATTATCTGACCTAAACGACTAATGGATGGATTTG
-----
+1 ThrArgLysGluGluMetGluArgGluLeuGlnThrProGlyArgAlaGlnIleSerAla
-----
241  ACTAGAAAGGAGGAGATGGAAAGGGAACCTTCAGACACCAGGCAGGGCTCAAATTTCTGCC
TGATCTTTCTCTCTACCTTTCCCTTGAAGTCTGTGGTCCGTCCCGAGTTTAAAGACGG
-----
+1 TyrArgPheHisPheCysArgMetSerTrpAlaGluAlaAsnSerGlnCysGlnThrGln
-----
301  TACAGGTTCCACTTCTGCCGCATGAGCTGGGCTGAAGCAAACAGCCAGTGCCAGACACAG
ATGTCCAAGGTGAAGACGGCGTACTCGACCCGACTTCGTTTGTCGGTCACGGTCTGTGTC
-----
+1 SerValProPheTrpArgArgValAspHisLeuLeuIleArgValMetLeuTyrGlnIle
-----
361  TCTGTACCTTTCTGGCGGAGGGTCGATCATCTATTAATAAGGGTCATGCTCTATCAGATT
AGACATGGAAAGACCGCTCCCAGCTAGTAGATAATTATTCCCAGTACGAGATAGTCTAA
-----
+1 SerGluGluValSerArgSerGluLeuArgSerPheLysPheLeuLeuGlnGluGluIle
-----
421  TCAGAAGAAGTGAGCAGATCAGAATTGAGGTCTTTTAAGTTTCTTTTGCAAGAGGAAATC
AGTCTTCTTCACTCGTCTAGTCTTAACTCCAGAAAATTCAAAGAAAACGTTCTCCTTTAG
-----
+1 SerLysCysLysLeuAspAspAspMetAsnLeuLeuAspIlePheIleGluMetGluLys
-----
481  TCCAAATGCAAACCTGGATGATGACATGAACCTGCTGGATATTTTCATAGAGATGGAGAAG
AGGTTTACGTTTGACCTACTACTGTACTTGGACGACCTATAAAAGTATCTCTACCTCTTC
-----
+1 ArgValIleLeuGlyGluGlyLysLeuAspIleLeuLysArgValCysAlaGlnIleAsn
-----
541  AGGGTCATCCTGGGAGAAGGAAAGTTGGACATCCTGAAAAGAGTCTGTGCCCAAATCAAC
TCCAGTAGGACCTCTTCTTTCAACCTGTAGGACTTTTCTCAGACACGGGTCTAGTTG
-----
+1 LysSerLeuLeuLysIleIleAsnAspTyrGluGluPheSerLysGlyGluGluLeuGln
-----
601  AAGAGCCTGCTGAAGATAATCAACGACTATGAAGAAATTCAGCAAGGCGAGGAGTTCTGTG
TTCTCGGACGACTCTCTTAAAGTCTGTGATACTTTTAAAGTCTTCCCTCTCTTAAAGCA

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Fig. 18B

CASPASE 8

```

+1 GlyValMetThrIleSerAspSerProArgGluGlnAspSerGluSerGlnThrLeuAsp
-----
661 GGGTAATGACAATCTCGGACTCTCCAAGAGAACAGGATAGTGAATCACAGACTTTGGAC
    CCCATTACTGTTAGAGCCTGAGAGGTTCTCTTGTCTATCACTTAGTGTCTGAAACCTG
-----
+1 LysValTyrGlnMetLysSerLysProArgGlyTyrCysLeuIleIleAsnAsnHisAsn
-----
721 AAAGTTTACCAAATGAAAAGCAAACCTCGGGGATACTGTCTGATCATCAACAATCACAAT
    TTTCAAATGGTTTACTTTTCGTTTGGAGCCCCTATGACAGACTAGTAGTTGTTAGTGTTA
-----
+1 PheAlaLysAlaArgGluLysValProLysLeuHisSerIleArgAspArgAsnGlyThr
-----
781 TTTGCAAAGCACGGGAGAAAGTGCCCAAACCTTCACAGCATTAGGGACAGGAATGGAACA
    AAACGTTTTCTGTGCCCTCTTTCACGGGTTTGAAGTGTCGTAATCCCTGTCTTACCTTGT
-----
+1 HisLeuAspAlaGlyAlaLeuThrThrThrPheGluGluLeuHisPheGluIleLysPro
-----
841 CACTTGGATGCAGGGGCTTTGACCACGACCTTTGAAGAGCTTCATTTTGAGATCAAGCCC
    GTGAACCTACGTCCCCGAAACTGGTGCTGGAACTTCTCGAAGTAAACTCTAGTTCGGG
-----
+1 HisHisAspCysThrValGluGlnIleTyrGluIleLeuLysIleTyrGlnLeuMetAsp
-----
901 CACCATGACTGCACAGTAGAGCAAATCTATGAGATTTTGAAATCTACCAACTCATGGAC
    GTGGTACTGACGTGTCATCTCGTTTAGATACTCTAAACTTTTAGATGGTTGAGTACCTG
-----
+1 HisSerAsnMetAspCysPheIleCysCysIleLeuSerHisGlyAspLysGlyIleIle
-----
961 CACAGTAACATGGACTGCTTCATCTGCTGTATCCTCTCCCATGGAGACAAGGGGCATCATC
    GTGTCATTGTACCTGACGAAGTAGACGACATAGGAGAGGGTACCTCTGTTCCCGTAGTAG
-----
+1 TyrGlyThrAspGlyGlnGluAlaProIleTyrGluLeuThrSerGlnPheThrGlyLeu
-----
1021 TATGGCACTGATGGACAGGAGGCCCCCATCTATGAGCTGACATCTCAGTTCAGTTGTTTG
    ATACCGTGACTACCTGTCTCCGGGGGTAGATACTCGACTGTAGAGTCAAGTGACCAAAC
-----
+1 LysCysProSerLeuAlaGlyLysProLysValPhePheIleGlnAlaCysGlnGlyAsp
-----
1081 AAGTGCCCTTCCCTTGCTGGAAAACCCAAAGTGTTTTTTATTCAGGCTTGTCAGGGGGAT
    TTCACGGGAAGGGAACGACCTTTTGGGTTTCACAAAAAATAAGTCCGAACAGTCCCCCTA
-----
+1 AsnTyrGlnLysGlyIleProValGluThrAspSerGluGluGlnProTyrLeuGluMet
-----
1141 AACTACCAGAAAGGTATACCTGTTGAGACTGATTCAGAGGAGCAACCCTATTTAGAAATG
    TTGATGGTCTTCCATATGGACAACCTCTGACTAAGTCTCCTCGTTGGGATAAATCTTTAC
-----
+1 AspLeuSerSerProGlnThrArgTyrIleProAspGluAlaAspPheLeuLeuGlyMet
-----
1201 GATTTATCATCACCTCAAACGAGATATATCCCGGATGAGGCTGACTTTCTGCTGGGGATG
    CTAAATAGTAGTGGAGTTTGCTCTATATAGGGCTACTCCGACTGAAAGACGACCCCTAC
-----
+1 AlaThrValAsnAsnCysValSerTyrArgAsnProAlaGluGlyThrTyrTyrIleGln
-----
1261 GCCACTGTGAATAACTGTGTTTCTTACCGAAACCCCTGCAGAGGGAACCTGGTACATCCAG
    CGGTGACACTTCTTGACACAAAGGATGGCTTTGGGACGTCCTCCTTGGACCATGTAGGTC

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Fig. 18C

CASPASE 8

```
+1 SerLeuCysGlnSerLeuArgGluArgCysProArgGlyAspAspIleLeuThrIleLeu
-----
1321 TCACTTTGCCAGAGCCTGAGAGAGCGATGTCCTCGAGGCGATGATATTCTCACCATCCTG
    AGTGAAACGGTCTCGGACTCTCTCGCTACAGGAGCTCCGCTACTATAAGAGTGGTAGGAC
-----
+1 ThrGluValAsnTyrGluValSerAsnLysAspAspLysLysAsnMetGlyLysGlnMet
-----
1381 ACTGAAGTGAACATATGAAGTAAGCAACAAGGATGACAAGAAAAACATGGGGAAACAGATG
    TGACTTCACTTGATACTTCATTCGTTGTTCTACTGTTCTTTTGTACCCCTTTGTCTAC
-----
+1 ProGlnProThrPheThrLeuArgLysLysLeuValPheProSerAsp
----->
1441 CCTCAGCCTACTTTCACACTAAGAAAAAACTTGTCTTCCCTTCTGATTGANN
    GGAGTCGGATGAAAGTGTGATTCTTTTTTTGAACAGAAGGGAAGACTAACTNN
-----
```

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Fig. 19A

CASPASE 9

```

+1 MetAspGluAlaAspArgArgLeuLeuArgArgCysArgLeuArgLeuValGluGluLeu
]-----
1  ATGGACGAAGCGGATCGGCGGCTCCTGCGGCGGTGCCGGCTGCGGCTGGTGGAGAGCTG
TACCTGCTTCGCCTAGCCGCCGAGGACGCCGCCACGGCCGACGCCGACCACCTTCTCGAC
-----
+1 GlnValAspGlnLeuTrpAspAlaLeuLeuSerSerGluLeuPheArgProHisMetIle
-----
61  CAGGTGGACCAGCTCTGGGACGCCCTGCTGAGCAGCGAGCTGTTTCAGGCCCATATGATC
GTCCACCTGGTCGAGACCCTGCGGGACGACTCGTCGCTCGACAAGTCCGGGGTATACTAG
-----
+1 GluAspIleGlnArgAlaGlySerGlySerArgArgAspGlnAlaArgGlnLeuIleIle
-----
121  GAGGACATCCAGCGGGCAGGCTCTGGATCTCGGCGGGATCAGGCCAGGCAGCTGATCATA
CTCCTGTAGGTCCCGTCCGAGACCTAGAGCCGCCCTAGTCCGGTCCGTCGACTAGTAT
-----
+1 AspLeuGluThrArgGlySerGlnAlaLeuProLeuPheIleSerCysLeuGluAspThr
-----
181  GATCTGGAGACTCGAGGGAGTCAGGCTCTTCCTTTGTTTCATCTCCTGCTTAGAGGACACA
CTAGACCTCTGAGCTCCCTCAGTCCGAGAAGGAAACAAGTAGAGGACGAATCTCCTGTGT
-----
+1 GlyGlnAspMetLeuAlaSerPheLeuArgThrAsnArgGlnAlaAlaLysLeuSerLys
-----
241  GGCCAGGACATGCTGGCTTCGTTTCTGCGAACTAACAGGCAAGCAGCAAAGTTGTCTGAAG
CCGGTCTGTACGACCGAAGCAAAGACGCTTGATTGTCCGTTTCGTCGTTTCAACAGCTTC
-----
+1 ProThrLeuGluAsnLeuThrProValValLeuArgProGluIleArgLysProGluVal
-----
301  CCAACCCTAGAAAACCTTACCCCAGTGGTGCTCAGACCAGAGATTCGCAAACCAGAGGTT
GGTTGGGATCTTTTGAATGGGGTCACCACGAGTCTGGTCTCTAAGCGTTTGGTCTCCAA
-----
+1 LeuArgProGluThrProArgProValAspIleGlySerGlyGlyPheGlyAspValGly
-----
361  CTCAGACCGGAAACACCCAGACCAGTGGACATTGGTTCTGGAGGATTTGGTGATGTCGGT
GAGTCTGGCCTTTGTGGGTCTGGTCACCTGTAACCAAGACCTCCTAAACCACTACAGCCA
-----
+1 AlaLeuGluSerLeuArgGlyAsnAlaAspLeuAlaTyrIleLeuSerMetGluProCys
-----
421  GCTCTTGAGAGTTTGAGGGGAAATGCAGATTTGGCTTACATCCTGAGCATGGAGCCCTGT
CGAGAACTCTCAAACTCCCCTTTACGTCTAAACCGAATGTAGGACTCGTACCTCGGGACA
-----
+1 GlyHisCysLeuIleIleAsnAsnValAsnPheCysArgGluSerGlyLeuArgThrArg
-----
481  GGCCACTGCCTCATTATCAACAATGTGAACCTTCTGCCGTGAGTCCGGGCTCCGCACCCGC
CCGGTGACGGAGTAATAGTTGTTACACTTGAAGACGGCACTCAGGCCCGAGGCGTGGGCG
-----
+1 ThrGlySerAsnIleAspCysGluLysLeuArgArgArgPheSerSerProHisPheMet
-----
541  ACTGGCTCCAACATCGACTGTGAGAAGTTGCGGCGTCGCTTCTCCTCGCCGCATTTTCATG
TGACCGAGGTTGTAGCTGACACTCTTCAACGCGCGAGCGAAGAGGAGCGGCGTAAAGTAC
-----
+1 ValGluValLysGlyAspLeuThrAlaLysLysMetValLeuAlaLeuLeuGluLeuAla
-----
601  GTGGAGGTGAAGGSCGACCTGACTGCCAAGAAAATGGTGGCTGGCTTTGCTGGAGCTGGCG
CACCTCCACTTCCCGCTGGACTGACGGTCTTTTACCACGACCGAAACGACCTCGACCGC

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Fig. 19B

CASPASE 9

```

+1 ArgGlnAspHisGlyAlaLeuAspCysCysValValValIleLeuSerHisGlyCysGln
-----
661 CGGCAGGACCACGGTGCTCTGGACTGCTGCGTGGTGGTCATTCTCTCTCACGGCTGTCAG
    GCCGTCCTGGTGCCACGAGACCTGACGACGCCACCAGTAAGAGAGAGTGCCGACAGTC
-----
+1 AlaSerHisLeuGlnPheProGlyAlaValTyrGlyThrAspGlyCysProValSerVal
-----
721 GCCAGCCACCTGCAGTTCCAGGGGCTGTCTACGGCACAGATGGATGCCCTGTGTCCGTC
    CGGTCGGTGGACGTCAAGGGTCCCCGACAGATGCCGTGTCTACCTACGGGACACAGCCAG
-----
+1 GluLysIleValAsnIlePheAsnGlyThrSerCysProSerLeuGlyGlyLysProLys
-----
781 GAGAAGATTGTGAACATCTTCAATGGGACCAGCTGCCCCAGCCTGGGAGGAAAGCCCAAG
    CTCTTCTAACACTTGTAGAAGTTACCCTGGTTCGACGGGGTCGGACCCTCCTTTTCGGGTTC
-----
+1 LeuPhePheIleGlnAlaCysGlyGlyGluGlnLysAspHisGlyPheGluValAlaSer
-----
841 CTCTTTTTTCATCCAGGCCTGTGGTGGGGAGCAGAAAGACCATGGGTTTGAGGTGGCCTCC
    GAGAAAAAGTAGGTCCGGACACCACCCCTCGTCTTTCTGGTACCCAAACTCCACCGGAGG
-----
+1 ThrSerProGluAspGluSerProGlySerAsnProGluProAspAlaThrProPheGln
-----
901 ACTTCCCCTGAAGACGAGTCCCCTGGCAGTAACCCCGAGCCAGATGCCACCCCGTTCCAG
    TGAAGGGGACTTCTGCTCAGGGGACCGTCATTGGGGCTCGGTCTACGGTGGGGCAAGGTC
-----
+1 GluGlyLeuArgThrPheAspGlnLeuAspAlaIleSerSerLeuProThrProSerAsp
-----
961 GAAGGTTTGGAGACCTTCGACCAGCTGGACGCCATATCTAGTTTGGCCACACCCAGTGAC
    CTTCCAAACTCCTGGAAGCTGGTCGACCTGCGGTATAGATCAAACGGGTGTGGGTCACTG
-----
+1 IlePheValSerTyrSerThrPheProGlyPheValSerTrpArgAspProLysSerGly
-----
1021 ATCTTTGTGTCTACTCTACTTTCCAGGTTTGTTCCTGGAGGGACCCCAAGAGTGGC
    TAGAAACACAGGATGAGATGAAAGGGTCCAAACAAAGGACCTCCCTGGGGTTCTCACCG
-----
+1 SerTrpTyrValGluThrLeuAspAspIlePheGluGlnTrpAlaHisSerGluAspLeu
-----
1081 TCCTGGTACGTTGAGACCCCTGGACGACATCTTTGAGCAGTGGGCTCACTCTGAAGACCTG
    AGGACCATGCAACTCTGGGACCTGCTGTAGAAACTCGTCACCCGAGTGAGACTTCTGGAC
-----
+1 GlnSerLeuLeuLeuArgValAlaAsnAlaValSerValLysGlyIleTyrLysGlnMet
-----
1141 CAGTCCCTCCTGCTTAGGGTCGCTAATGCTGTTTCGGTGAAAGGGATTTATAAACAGATG
    GTCAGGGAGGACGAATCCGAGCGATTACGACAAAGCCACTTTCCCTAAATATTTGTCTAC
-----
+1 ProGlyCysPheAsnPheLeuArgLysLysLeuPhePheLysThrSer
-----
1201 CCTGGTTGCTTTAATTTCCCTCGGAAAAAACTTTTCTTTAAAACATCATAAN
    GGACCAACGAAATTAAAGGAGGCCCTTTTGTGAAAAGAAATTTTGTAGTATTN

```


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Fig. 20A

CASPASE 10

```

+1 MetLysSerGlnGlyGlnHisTrpTyrSerSerSerAspLysAsnCysLysValSerPhe
]-----
1  ATGAAATCTCAAGGTCAACATTGGTATTCCAGTTCAGATAAAACTGTAAAGTGAGCTTT
   TACTTTAGAGTTCCAGTTGTAACCATAAGGTCAAGTCTATTTTTGACATTTCACTCGAAA
+1 ArgGluLysLeuLeuIleIleAspSerAsnLeuGlyValGlnAspValGluAsnLeuLys
-----
61  CGTGAGAAGCTTCTGATTATTGATTCAAACCTGGGGTCCAAGATGTGGAGAACCTCAAG
   GCACTCTTCGAAGACTAATAACTAAGTTTGGACCCCCAGGTTCTACACCTCTTGGAGTTC
+1 PheLeuCysIleGlyLeuValProAsnLysLysLeuGluLysSerSerSerAlaSerAsp
-----
121  TTTCTCTGCATAGGATTGGTCCCCAACAAGAAGCTGGAGAAGTCCAGCTCAGCCTCAGAT
   AAAGAGACGTATCCTAACCAGGGGTGTTCTTCGACCTCTTCAGGTCGAGTCGGAGTCTA
+1 ValPheGluHisLeuLeuAlaGluAspLeuLeuSerGluGluAspProPhePheLeuAla
-----
181  GTTTTTGAACATCTCTTGGCAGAGGATCTGCTGAGTGAGGAAGACCCTTTCTTCTGGCA
   CAAAAACTTGTAGAGAACCGTCTCCTAGACGACTCACTCCTTCTGGGAAAGAAGGACCGT
+1 GluLeuLeuTyrIleIleArgGlnLysLysLeuLeuGlnHisLeuAsnCysThrLysGlu
-----
241  GAACTCCTCTATATCATACGGCAGAAGAAGCTGCTGCAGCACCTCAACTGTACCAAAGAG
   CTTGAGGAGATATAGTATGCCGTCTTCTTCGACGACGTCGTGGAGTTGACATGGTTTCTC
+1 GluValGluArgLeuLeuProThrArgGlnArgValSerLeuPheArgAsnLeuLeuTyr
-----
301  GAAGTGGAGCGACTGCTGCCACCCGACAAAGGGTTTCTCTGTTTAGAAACCTGCTCTAC
   CTTACCTCGCTGACGACGGGTGGGCTGTTTCCCAAAGAGACAAATCTTTGGACGAGATG
+1 GluLeuSerGluGlyIleAspSerGluAsnLeuLysAspMetIlePheLeuLeuLysAsp
-----
361  GAACTGTCAGAAGGCATTGACTCAGAGAACTTAAAGGACATGATCTTCCTTCTGAAAGAC
   CTTGACAGTCTTCCGTAAGTGAAGTCTCTTGAATTTCTGTACTAGAAGGAAGACTTTCTG
+1 SerLeuProLysThrGluMetThrSerLeuSerPheLeuAlaPheLeuGluLysGlnGly
-----
421  TCGCTTCCCAAACTGAAATGACCTCCCTAAGTTTCTGGCATTCTAGAGAAACAAGGT
   AGCGAAGGGTTTTGACTTTACTGGAGGGATTCAAAGGACCGTAAAGATCTCTTTGTTCCA
+1 LysIleAspGluAspAsnLeuThrCysLeuGluAspLeuCysLysThrValValProLys
-----
481  AAAATAGATGAAGATAATCTGACATGCCTGGAGGACCTCTGCAAAACAGTTGTACCTAAA
   TTTTATCTACTTCTATTAGACTGTACGGACCTCTGGAGACGTTTTGTCAACATGGATTT
+1 LeuLeuArgAsnIleGluLysTyrLysArgGluLysAlaIleGlnIleValThrProPro
-----
541  CTTTTGAGAAACATAGAGAAATACAAAAGAGAGAAAGCTATCCAGATAGTGACACCTCT
   GAAAACCTCTTTGTATCTCTTTATGTTTTCTCTCTTTCGATAGGTCTATCACTGTGGAGGA
+1 ValAspLysGluAlaGluSerTyrGlnGlyGluGluGluLeuValSerGlnThrAspVal
-----
601  GTAGACAAGGAAGCCGAGTTCGTATCAAGGAGAGGAAGAACTAGTTTCCCAAACAGATGTT
   CATCTGTTCTTGGGCTCAGCATAGTTCTCTCTCTCTCTGATCAAAGGTTTGTCTACAA

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Fig. 20B

CASPASE 10

+1 LysThrPheLeuGluAlaLeuProArgAlaAlaValTyrArgMetAsnArgAsnHisArg

 661 AAGACATTCTTGGAGCCTTACCGAGGGCAGCTGTGTACAGGATGAATCGGAACCACAGA
 TTCTGTAAGAACCTTCGGAATGGCTCCCGTCGACACATGTCCTACTTAGCCTTGGTGTCT

 +1 GlyLeuCysValIleValAsnAsnHisSerPheThrSerLeuLysAspArgGlnGlyThr

 721 GGCCTCTGTGTCATTGTCAACAACCACAGCTTTACCTCCCTGAAGGACAGACAAGGAACC
 CCGGAGACACAGTAACAGTTGTTGGTGTGAAATGGAGGGACTTCCTGTCTGTTCCCTTGG

 +1 HisLysAspAlaGluIleLeuSerHisValPheGlnTrpLeuGlyPheThrValHisIle

 781 CATAAAGATGCTGAGATCCTGAGTCATGTGTTCCAGTGGCTTGGGTTCACAGTGCATATA
 GTATTTCTACGACTCTAGGACTCAGTACACAAGGTCACCGAACCCAAGTGTACGTATAT

 +1 HisAsnAsnValThrLysValGluMetGluMetValLeuGlnLysGlnLysCysAsnPro

 841 CACAATAATGTGACGAAAGTGGAAATGGAGATGGTCCTGCAGAAGCAGAAGTGCAATCCA
 GTGTTATTACACTGCTTTACCTTTACCTCTACCAGGACGTCTTCGTCTTCACGTTAGGT

 +1 AlaHisAlaAspGlyAspCysPheValPheCysIleLeuThrHisGlyArgPheGlyAla

 901 GCCCATGCCGACGGGGACTGCTTCGTGTTCTGTATTCTGACCCATGGGAGATTTGGAGCT
 CGGGTACGGCTGCCCTGACGAAGCACAAGACATAAGACTGGGTACCCTCTAAACCTCGA

 +1 ValTyrSerSerAspGluAlaLeuIleProIleArgGluIleMetSerHisPheThrAla

 961 GTCTACTCTTCGGATGAGGCCCTCATTCCCATTCGGGAGATCATGTCTCACTTCACAGCC
 CAGATGAGAAGCCTACTCCGGGAGTAAGGGTAAGCCCTCTAGTACAGAGTGAAGTGTGCG

 +1 LeuGlnCysProArgLeuAlaGluLysProLysLeuPhePheIleGlnAlaCysGlnGly

 1021 CTGCAGTGCCCTAGACTGGCTGAAAAACCTAAACTCTTTTTTCATCCAGGCCTGCCAAGGT
 GACGTCACGGGATCTGACCGACTTTTGGATTTGAGAAAAAGTAGGTCCGGACGGTTCCA

 +1 GluGluIleGlnProSerValSerIleGluAlaAspAlaLeuAsnProGluGlnAlaPro

 1081 GAAGAGATACAGCCTTCCGTATCCATCGAAGCAGATGCTCTGAACCCTGAGCAGGCACCC
 CTTCTCTATGTGCGAAGGCATAGGTAGCTTCGTCTACGAGACTTGGGACTCGTCCGTGGG

 +1 ThrSerLeuGlnAspSerIleProAlaGluAlaAspPheLeuLeuGlyLeuAlaThrVal

 1141 ACTTCCCTGCAGGACAGTATTCTGCCGAGGCTGACTTCCTACTTGGTCTGGCCACTGTC
 TGAAGGGACGTCCTCTCATAAGGACGGCTCCGACTGAAGGATGAACCAGACCGGTGACAG

 +1 ProGlyTyrValSerPheArgHisValGluGluGlySerTrpTyrIleGlnSerLeuCys

 1201 CCAGGCTATGTATCCTTTCCGGCATGTGGAGGAAGGCAGCTGGTATATTCAGTCTCTGTGT
 GGTCCGATACATAGGAAAGCCGTACACCTCCTCCGTCCGACCATATAAGTCAGAGACACA

 +1 AsnHisLeuLysLysLeuValProArgHisGluAspIleLeuSerIleLeuThrAlaVal

 1261 AATCATCTGAAGAAATTGGTCCCAAGACATGAAGACATCTTATCCATCCTCACTGCTGTC
 TTAGTAGACTTCTTAAACAGGGTCTGTACTTCTGTAGAATAGGTAGGASTGACGACAG

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Fig. 20C

CASPASE 10

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+1 AsnAspAspValSerArgArgValAspLysGlnGlyThrLysLysGlnMetProGlnPro
-----
1321 AACGATGATGTGAGTCGAAGAGTGGACAAACAGGGAACAAAGAAACAGATGCCCCAGCCT
    TTGCTACTACACTCAGCTTCTCACCTGTTTGTCCCTTGTTTCTTTGTCTACGGGGTCGGA
-----
+1 AlaPheThrLeuArgLysLysLeuValPheProValProLeuAspAlaLeuSerIle
----->
1381 GCTTTCACACTAAGGAAAAAACTAGTATTCCCTGTGCCCTGGATGCACTTTCAATATAG
    CGAAAGTGTGATTCTTTTGTATCATAAGGGACACGGGGACCTACGTGAAAGTTATATC
-----

```

Fig. 21A

Rev-caspase-3

```

Met Ile Glu Thr Asp Ser Gly Val Asp Asp Asp Met Ala Cys His Lys Ile Pro
Val Glu Ala Asp Phe Leu Tyr Ala Tyr Ser Thr Ala Pro Gly Tyr Tyr Ser Trp
Arg Asn Ser Lys Asp Gly Ser Trp Phe Ile Gln Ser Leu Cys Ala Met Leu Lys
Gln Tyr Ala Asp Lys Leu Glu Phe Met His Ile Leu Thr Arg Val Asn Arg Lys
Val Ala Thr Glu Phe Glu Ser Phe Ser Phe Asp Ala Thr Phe His Ala Lys Lys
Gln Ile Pro Cys Ile Val Ser Met Leu Thr Lys Glu Leu Tyr Phe Tyr His Asp
Glu Val Asp Gly Gly Ser Pro Met Glu Asn Thr Glu Asn Ser Val Asp Ser Lys
Ser Ile Lys Asn Leu Glu Pro Lys Ile Ile His Gly Ser Glu Ser Met Asp Ser
Gly Ile Ser Leu Asp Asn Ser Tyr Lys Met Asp Tyr Pro Glu Met Gly Leu Cys
Ile Ile Ile Asn Asn Lys Asn Phe His Lys Ser Thr Gly Met Thr Ser Arg Ser
Gly Thr Asp Val Asp Ala Ala Asn Leu Arg Glu Thr Phe Arg Asn Leu Lys Tyr
Glu Val Arg Asn Lys Asn Asp Leu Thr Arg Glu Glu Ile Val Glu Leu Met Arg
Asp Val Ser Lys Glu Asp His Ser Lys Arg Ser Ser Phe Val Cys Val Leu Leu
Ser His Gly Glu Glu Gly Ile Ile Phe Gly Thr Asn Gly Pro Val Asp Leu Lys
Lys Ile Thr Asn Phe Phe Arg Gly Asp Arg Cys Arg Ser Leu Thr Gly Lys Pro
Lys Leu Phe Ile Ile Gln Ala Cys Arg Gly Thr Glu Leu Asp Cys Gly Ile Glu
Thr Asp

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Fig. 21B

Uncleavable Rev-caspase-3

Met	Ile	Glu	Thr	Asp	Ser	Gly	Val	Asp	Asp	Asp	Met	Ala	Cys	His	Lys	Ile	Pro
Val	Glu	Ala	Asp	Phe	Leu	Tyr	Ala	Tyr	Ser	Thr	Ala	Pro	Gly	Tyr	Tyr	Ser	Trp
Arg	Asn	Ser	Lys	Asp	Gly	Ser	Trp	Phe	Ile	Gln	Ser	Leu	Cys	Ala	Met	Leu	Lys
Gln	Tyr	Ala	Asp	Lys	Leu	Glu	Phe	Met	His	Ile	Leu	Thr	Arg	Val	Asn	Arg	Lys
Val	Ala	Thr	Glu	Phe	Glu	Ser	Phe	Ser	Phe	Asp	Ala	Thr	Phe	His	Ala	Lys	Lys
Gln	Ile	Pro	Cys	Ile	Val	Ser	Met	Leu	Thr	Lys	Glu	Leu	Tyr	Phe	Tyr	His	Gly
Ser	Pro	Met	Glu	Asn	Thr	Glu	Asn	Ser	Val	Ala	Ser	Lys	Ser	Ile	Lys	Asn	Leu
Glu	Pro	Lys	Ile	Ile	His	Gly	Ser	Glu	Ser	Met	Ala	Ser	Gly	Ile	Ser	Leu	Asp
Asn	Ser	Tyr	Lys	Met	Asp	Tyr	Pro	Glu	Met	Gly	Leu	Cys	Ile	Ile	Ile	Asn	Asn
Lys	Asn	Phe	His	Lys	Ser	Thr	Gly	Met	Thr	Ser	Arg	Ser	Gly	Thr	Asp	Val	Asp
Ala	Ala	Asn	Leu	Arg	Glu	Thr	Phe	Arg	Asn	Leu	Lys	Tyr	Glu	Val	Arg	Asn	Lys
Asn	Asp	Leu	Thr	Arg	Glu	Glu	Ile	Val	Glu	Leu	Met	Arg	Asp	Val	Ser	Lys	Glu
Asp	His	Ser	Lys	Arg	Ser	Ser	Phe	Val	Cys	Val	Leu	Leu	Ser	His	Gly	Glu	Glu
Gly	Ile	Ile	Phe	Gly	Thr	Asn	Gly	Pro	Val	Asp	Leu	Lys	Lys	Ile	Thr	Asn	Phe
Phe	Arg	Gly	Asp	Arg	Cys	Arg	Ser	Leu	Thr	Gly	Lys	Pro	Lys	Leu	Phe	Ile	Ile
Gln	Ala	Cys	Arg	Gly	Thr	Glu	Leu	Asp	Cys	Gly	Ile	Glu	Thr	Asp			

Fig. 21C

> Rev-caspase-6

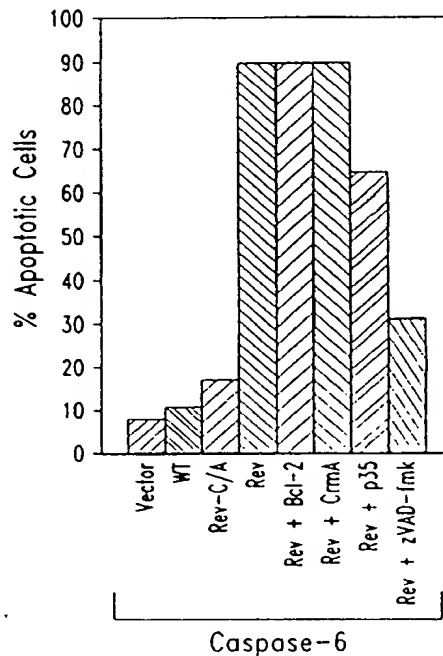
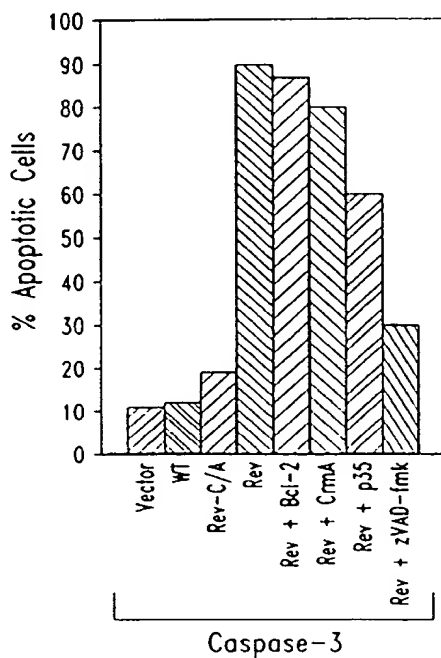
Met	Val	Glu	Ile	Asp	Ala	Ala	Ser	Val	Tyr	Thr	Leu	Pro	Ala	Gly	Ala	Asp	Phe
Leu	Met	Cys	Tyr	Ser	Val	Ala	Glu	Gly	Tyr	Tyr	Ser	His	Arg	Glu	Thr	Val	Asn
Gly	Ser	Trp	Tyr	Ile	Gln	Asp	Leu	Cys	Glu	Met	Leu	Gly	Lys	Tyr	Gly	Ser	Ser
Leu	Glu	Phe	Thr	Glu	Leu	Leu	Thr	Leu	Val	Asn	Arg	Lys	Val	Ser	Gln	Arg	Arg
Val	Asp	Phe	Cys	Lys	Asp	Pro	Ser	Ala	Ile	Gly	Lys	Lys	Gln	Val	Pro	Cys	Phe
Ala	Ser	Met	Leu	Thr	Lys	Lys	Leu	His	Phe	Phe	Pro	Lys	Ser	Asn	Leu	Glu	His
His	His	His	His	His	Val	Glu	Ile	Asp	Gly	Gly	Ser	Pro	Met	Ser	Ser	Ala	Ser
Gly	Leu	Arg	Arg	Gly	His	Pro	Ala	Gly	Gly	Glu	Glu	Asn	Met	Thr	Glu	Thr	Asp
Ala	Phe	Tyr	Lys	Arg	Glu	Met	Phe	Asp	Pro	Ala	Glu	Lys	Tyr	Lys	Met	Asp	His
Arg	Arg	Arg	Gly	Ile	Ala	Leu	Ile	Phe	Asn	His	Glu	Arg	Phe	Phe	Trp	His	Leu
Thr	Leu	Pro	Glu	Arg	Arg	Gly	Thr	Cys	Ala	Asp	Arg	Asp	Asn	Leu	Thr	Arg	Arg
Phe	Ser	Asp	Leu	Gly	Phe	Glu	Val	Lys	Cys	Phe	Asn	Asp	Leu	Lys	Ala	Glu	Glu
Leu	Leu	Leu	Lys	Ile	His	Glu	Val	Ser	Thr	Val	Ser	His	Ala	Asp	Ala	Asp	Cys
Phe	Val	Cys	Val	Phe	Leu	Ser	His	Gly	Glu	Gly	Asn	His	Ile	Tyr	Ala	Tyr	Asp
Ala	Lys	Ile	Glu	Ile	Gln	Thr	Leu	Thr	Gly	Leu	Phe	Lys	Gly	Asp	Lys	Cys	His
Ser	Leu	Val	Gly	Lys	Pro	Lys	Ile	Phe	Ile	Ile	Gln	Ala	Cys	Arg	Gly	Asn	Gln
His	Asp	Val	Pro	Val	Ile	Pro	Leu	Asp	Val	Val	Asp						



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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			(43) International Publication Date: 15 July 1999 (15.07.99)
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(71) Applicant: THOMAS JEFFERSON UNIVERSITY [US/US]: 1020 Locust Street, Philadelphia, PA 19107-6799 (US).			
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(74) Agents: MAKI, David, J. et al.: Seed and Berry LLP, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).			
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(54) Title: RECOMBINANT, ACTIVE CASPASES AND USES THEREOF



(57) Abstract

Rev-caspases comprising a primary product in which the small subunit is N terminal to the large subunit are provided. Rev-caspases are used for screening and identifying caspase inhibitors and enhancers. Rev-caspase genes can be delivered to cells for gene therapy.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/00632

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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Remark: Although claims 35 and 36
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
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because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

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- ☐ No protest accompanied the payment of additional search fees

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9531535	A	23-11-1995	US 5492824 A	20-02-1996
			CA 2189581 A	23-11-1995
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			JP 10503084 T	24-03-1998
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			JP 5227961 A	07-09-1993

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INTERNATIONAL SEARCH REPORT

International Application No.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/57 C12N9/64 C12N1/21 C12N5/10 C12Q1/37
A61K48/00

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 31535 A (BASF AG) 23 November 1995 (1995-11-23) page 2, paragraph 3 - page 5, paragraph 2; examples	1-36
A	EP 0 533 350 A (MRCK & CO., INC.) 24 March 1993 (1993-03-24) page 5, line 3 - page 13, line 2; examples 23-31	1-36

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☒ Further documents are listed in the continuation of box C☒ Patent family members are listed in annex

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P.X	<p>SRINIVASA M. SRINIVASULA ET AL.: "Generation of constitutively active recombinant Caspases-3 and -6 by rearrangement of their subunits" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 17, 24 April 1998 (1998-04-24), pages 10107-10111, XP002111125 MD US abstract page 10108, left-hand column, paragraph 2 - page 10110, right-hand column, paragraph 3</p> <p>-----</p>	1-36

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